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#### I. INTRODUCTION

This report describes the results of studies and experiments on sample collection and processing carried out during the period from October 2, 1962, to June 2, 1963, under a Contract Supplement to JPL Contract No. 950123, Microscopic System for Mars Study Program. The primary objective of this experimental design-study program was the construction and testing of a pneumatic sample-collection unit for use with a microscope or some other Martian biological experiment. The secondary objective was the study and determination of the most suitable liquid and flotation procedure for efficiently separating and concentrating microbiological particles from mineral particles, to facilitate their being viewed by a microscopic system.

The experimental sampler unit's design was based on

- 1) Concepts which evolved during the Mars microscope system feasibility study;
- 2) Results from a preliminary experimental design study;
- 3) Reliability requirements;
- 4) Imposed (or desired) maximum sampler unit weight, power, and size;
- 5) Desired sample size and sampling time;
- 6) Earth and simulated Martian environmental conditions at which the unit must be tested:
- 7) Space conditions in which the unit must be transported;
- 8) Actual Martian conditions in which the unit is expected to operate.

A sampler unit which generally satisfied all of the above criteria resulted after certain assumptions were made and certain compromises were incorporated into its design. By contractual agreement, our method of sampling was to emphasize pneumatic techniques. The type of preliminary information required before designing a prototype unit was therefore 1) the

best method of aerosolizing ground particulate material; 2) the size, size distribution, and concentration of microorganisms in an aerosolized cloud; 3) the best methods of transporting and collecting air-borne particles in the desired size; and 4) the system's power, weight, and volume requirements. Information of this type was obtained both by calculation and by experimentation, and was used to establish the basic sampler's design criteria.

The design, construction, and testing of a preliminary sampler was followed by design modification, construction, and testing of a prototype sampler unit, which was delivered to JPL. Although this prototype unit is far from a flight model, it does satisfy the requirements previously listed. For example, the unit built for Earth testing sampled at a rate sufficient to provide a 1-gram sample in 6 minutes, while traversing a dry sandy surface. The Martian sampler unit, in its packaged state, will occupy about 1/20 cu ft, would have a flight weight of under 2 lb, and would require about 5 w of power to operate in a Martian environment. Furthermore, this unit can be adapted to most biological experiments or to a density-processing device, can be remotely operated, and can reach out 10 ft from the sampler housing to acquire the sample.

The study of sample-processing techniques has resulted in our finding a satisfactory flotation liquid and in our working out a laboratory flotation procedure which appears amenable to automation, compatible with the sampling unit, justifiable for a microscopic system, and which meets the power, weight, and volume requirements of a Martian system. Sufficient laboratory data have been obtained both to design a sample-processing system for the sampling unit and to predict the performance obtainable from such a system under a given set of conditions. For example: 1 gram of dust provided by the sampling unit can be collected in 3 ml of fluorochemical, centrifuged at 24,000 rpm for 5 min in a 2-inch diameter chamber, overlayed with less than 1 ml of water, and agitated slightly to give a relatively clear (90 percent light transmission) overlay fraction containing from 10 to 90 percent (depending on type of organism) of the microbiological material.

A brief summary of the study results and highlights, together with conclusions and recommendations constitute the body of this report.

A comprehensive account of the sampling unit's design and of the biological support study are contained in Appendixes A and B. A complete set of shop drawings of the sampling unit are included as Appendix C.

#### II. SUMMARY AND CONCLUSIONS

#### A. Calculations

The information required to establish proper design parameters for a pneumatic sample-collection unit includes flow rate and pressure-drop characteristics of the system, performance characteristics of the blower, and the quantity and size range of the particles to be collected. For this program, the flow and pressure-drop characteristics of several systems in the approximate range of interest were calculated. The final choice of system parameters was then based on the performances of available blowers and on the minimum particle size that must be collected to assure acquisition of a majority of viable organisms initially present in the soil samples. Computations were made for standard atmospheres of both Mars and Earth. Results for the Earth computations are presented in Figure 1. These data indicate the total pressure drop versus air-flow rate and collected particlesize range for the basic collection system, which is shown in Figure 2.

The performance curves of two blowers are superimposed on the operating curves of Figure 1. The intersection of the curves indicates the particle size range which the system will collect as a function of flow rate. Obviously, these blowers do not develop sufficient pressure to operate a system designed to collect particles less than about 4 microns in diameter.

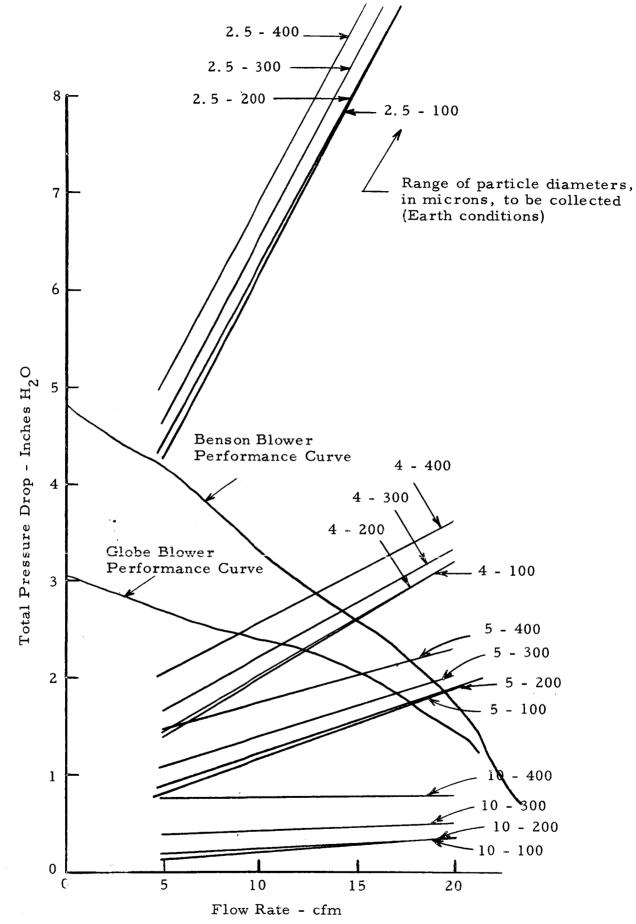


Figure 1. Total Pressure Drop of Collection System versus Flow Rate at Earth Conditions

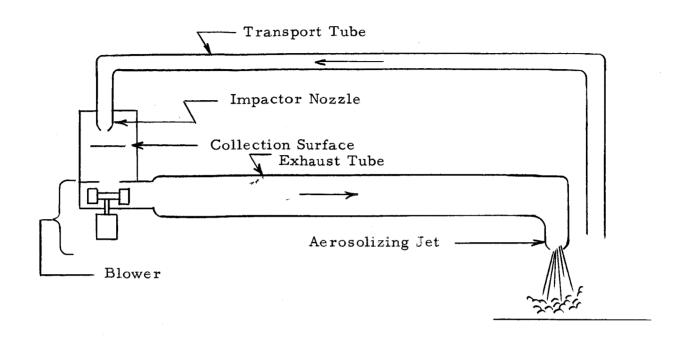


Figure 2. Pneumatic Particle Collection System
Considered for Pressure Drop Calculations

#### B. Aerosolization Studies

Since blowers having the desired physical dimensions and power consumption could collect the larger particles from fairly large volumes of air, the percentage of aerosolized viable organisms associated with these larger particles (larger than about 4 microns) was determined. The results of several experiments conducted to determine this relationship in aerosolized dusts are listed in Table I. These data show that a majority of the organisms aerosolized from dry unfertile soils are associated with particles larger than 4 microns. Because a two-fold decrease in the collected particle diameter requires about a four-fold increase in the system's pressure drop or power, the remaining fraction of the organisms are prohibitively expensive (i. e., it requires much less power to collect 50 percent of the organisms from 10 cu ft than to collect 100 percent from 5 cu ft).

Table I. Microorganism-Particle Size Relationship Obtained by Aerosolizing Various Dry Soils

Type of	Count per Particle Size Interval						
Soil	>10 µ	6-10 д	4-6 μ	2-4 μ	1-2 μ	< 1 μ	Count
Sand No. 1	47	24	15	11	5	0	102
Sand No. 2	420	160	97	24	14	20	735
Gravel	84	221	185	91	13	10	604
Asphalt	31	15	7	6	12	5	76
Mulch	53	72	61	93	326	516	1121
Field Soil	48	36	18	16	5	8	131

## C. Component Testing

A preliminary test program was performed concurrently with the calculation phase to evaluate commercially-available hardware and to test promising design ideas. One significant result of this program was the acquisition of a small, high-speed motor, which, when teamed with a previouslyacquired vane-axial blower, provided sufficient pressure and flow capacity
to operate a collection system at Martian atmospheric conditions while
drawing only 5 w of power. This is well within the power-requirement limits
set by the original work statement.

A second point of significance was the development of a very satisfactory aerosolizer-transport tube assembly. These tubes were fabricated by covering helical coil springs with lightweight coated fabric or Mylar. The 10-ft long tubes, when packaged in their normal concentric operating positions, can be compressed into a package less than 2.5 inches in diameter and 2.5 inches long. This was a major factor in achieving minimum package volume and weight.

Another important concept was the utilization of the blower's effluent to inflate a pneumatic device. Through proper design, blower pressure can be utilized at the expense of very little power to pressurize an inflatable structure suitable for use as an activation, orientation, or locomotion device. This idea led to using inflatable tires for a mobile aerosolizer-sampler unit.

#### D. Design Parameters

The preliminary calculation and test phase culminated with establishment of the parameters (listed in Table II) that were needed to design a collection system for Earth conditions capable of collecting particles with diameters in the size range from 5 to 400 microns.

Table II. Design Parameters for a Collector Operating under Earth Conditions

Flow Rate	15 cfm
Pressure Head	2 inches H <sub>2</sub> O
Transport Tube Diameter	1.3 inches
Effluent Tube Diameter	2.3 inches
Impactor Nozzle Diameter	2.0 cm

#### E. Design Study

We were uncertain about how well the sampling unit would function. A breadboard model was therefore built and tested before the prototype sampler was designed. This also allowed the hardware items and design ideas acquired in the preliminary phase to be combined and evaluated under simulated field conditions. The sampling unit was built to the specification called out in the previous section and employed a Benson vane-axial blower as its prime air mover. The aerosolizer-collector assembly was mobilized by use of inflatable tires that were driven by a small electric motor. The whole unit, including the 10-ft long tubes (when expanded), was packaged in a 10-inch long by 3-inch diameter cylinder.

Several tests were conducted to establish the performance characteristics of this collector at earth conditions. The unit was found to be capable of collecting I gram of particulate material in approximately 6 minutes. Other tests indicated that this represented approximately 15 percent of the particles introduced at the collection nozzle. Because the total quantity collected was substantially more than originally anticipated, it did not appear necessary to expend a great deal of effort to improve over-all efficiency.

The unit was also evaluated with respect to such mechanical functions as packaging, ejection from the canister, sample handling, aerosolizer mobility, and aerosolizer reliability. This qualitative evaluation demonstrated the need for additional design work on the aerosolizer's ejection mechanism and the mobile aerosolizer unit.

### F. Sampling-Unit Design

A prototype sampling system was designed and fabricated from information gathered in the preliminary work. During and after assembly, a number of newly-designed components were tested for mechanical performance and modified where necessary. Figure 3 is a photograph of the system showing the aerosolizer, transport tubes, cover and extraction assembly, and the main housing. Figure 4 is an assembly drawing of the unit. An exploded view of the aerosolizer is presented as Figure 5.

In operation, a motor in the cover drives two jack screws by a chain and sprocket arrangement. The screws, operating through ball nuts attached to the housing, force the cover away from the housing. Two fingers attached to the cover extract the aerosolizer and transport tube and drop them to the ground.

Effluent air from the blower (the outside tube) inflates the tires and is then directed against the ground through the aerosolizer nozzle. Aerosolized dust is drawn up the inner tube and collected by impaction or filtration in the collection stage. An impactor stage is the desired and intended method for particle collection, but for demonstration purposes the impactor has been replaced by a filter unit. The aerosolizer assembly is driven slowly over the ground by an electric motor. No means or device is included in the present sampler for transporting the collected sample to a processing or biological test chamber.

#### G. Sampler Unit Evaluation

As mentioned, the prototype sampler was designed for test and evaluation at earth conditions and was found to operate satisfactorily at those conditions. The ultimate objective, of course, is to design and build a unit that will operate satisfactory under actual Martian conditions. Because of the difference in Martian and Earth gravities, the unit could only be tested at a simulated Martian air density (100 mb pressure) and an Earth gravity.

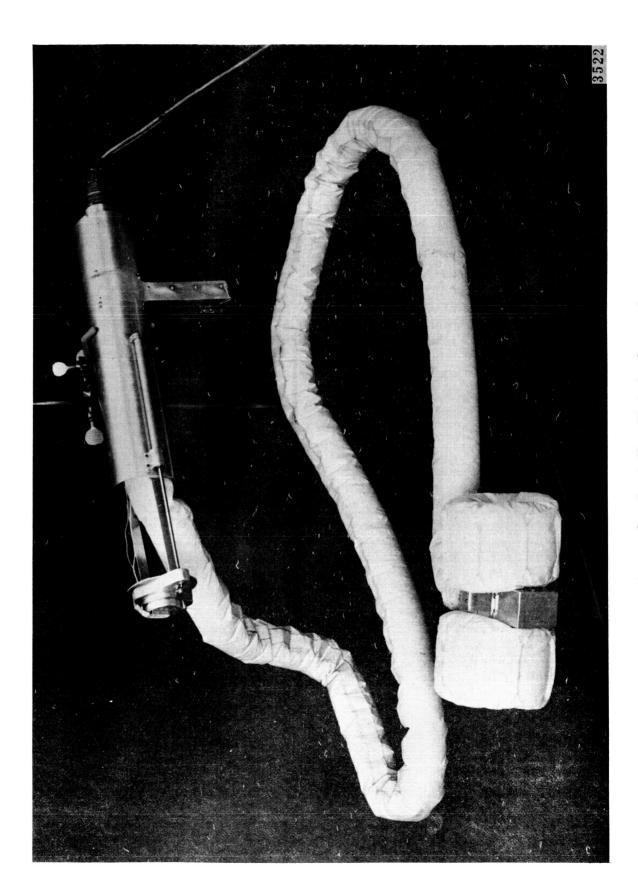


Figure 3. Sampling Unit in Operation

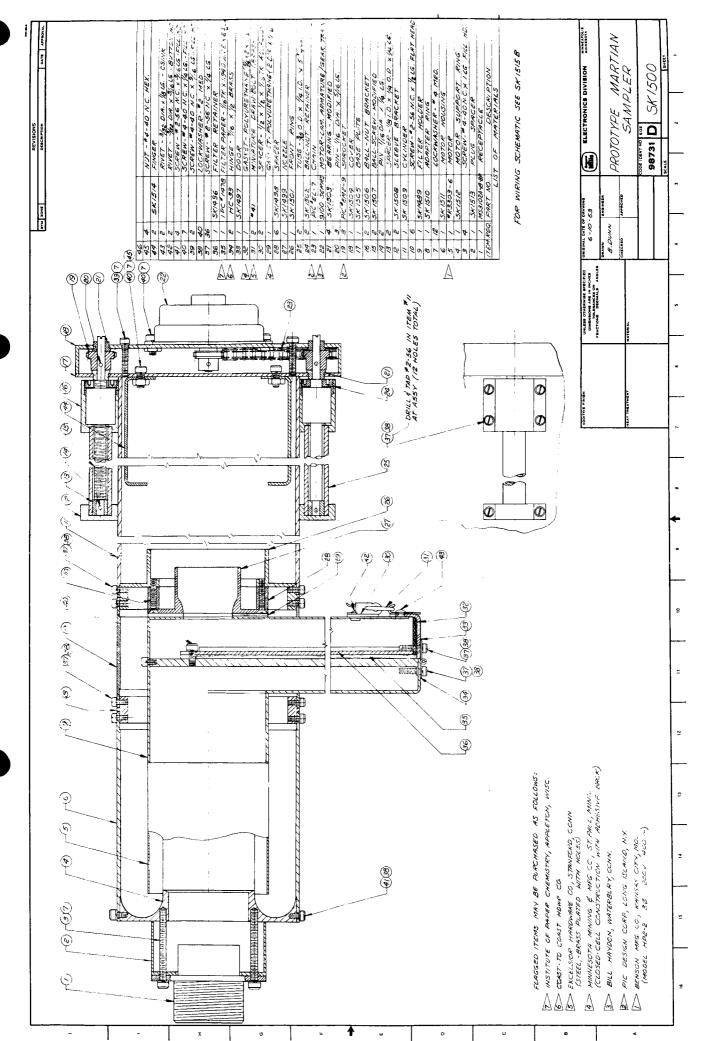


FIGURE 4

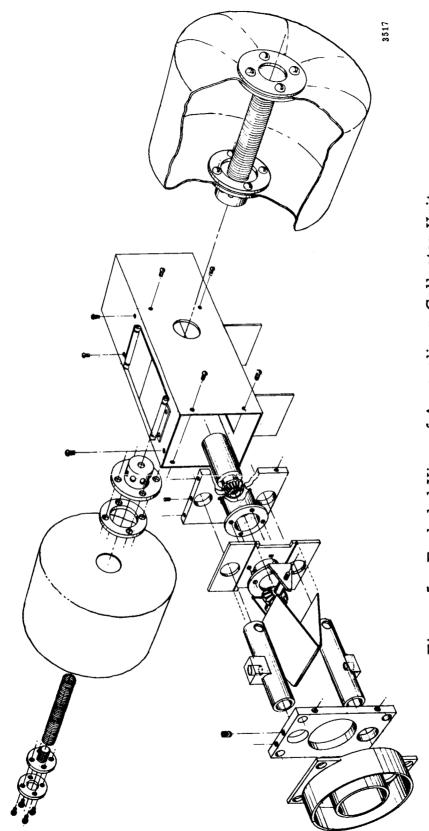


Figure 5. Exploded View of Aerosolizer-Collector Unit

The test format paralleled as far as possible that used in testing the breadboard model at standard conditions, to allow some type of data comparison. These data are presented graphically in Figure 6. Depending on the type of soil, the prototype collected from 5 to 25 percent as much soil at 1/10 atmosphere as the breadboard model did at standard conditions. This reduced performance was expected for two reasons. First, a unit designed to operate on Earth is markedly different from one designed to work on Mars. Secondly, a unit designed to work on Mars but tested on Earth, would collect about one-third the dust sample on Earth because of the three-fold higher gravity. When the gravity difference plus the off-design operation of the Earth unit in a Martian air density is considered, the collection rate for a sampler on Mars would be as great or greater than that for an Earth unit at standard conditions.

Low power requirement for a sampler operating on Mars was exemplified by reduced-pressure testing of the unit fitted with a motor-blower designed for Martian conditions. The measured motor power input was about 5 w.

#### H. Biological Support Study

During the course of this project extension, the biological laboratory carried out several investigations directly applicable to the design of sample-processing apparatus.

One area which received fairly intense investigation involved the evaluation of various high-density fluids that could be used for separating biological material from dusts and soils by flotation. Twenty-eight fluids were tested against the criteria that density flotation should simultaneously accomplish both separation of biological from nonbiological particles (purification) and retention of the biological fraction in a relatively small portion of liquid (concentration). These criteria are in addition to those imposed on the fluids by their intended use in the Martian environment.

A number of different laboratory techniques were used to evaluate the fluids including culturing and microscopy.

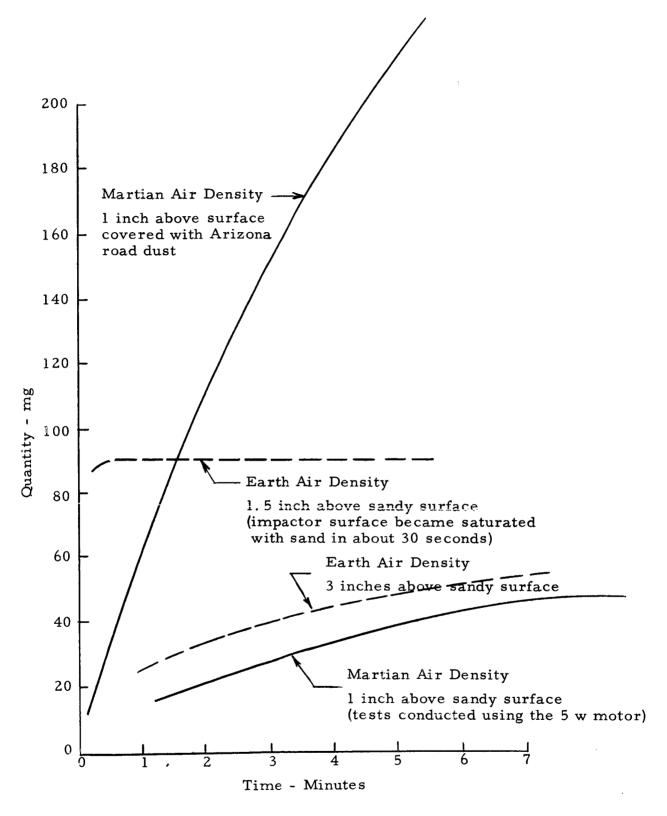


Figure 6. Performance of the Earth Particle Collection System at Earth and Martian Air Density

The results of these tests suggest that there are several feasible ways in which flotation may be incorporated into a purification-concentration system for biological particles. For large entities such as rust spores, simple mixing of the aerosol with (or collecting the aerosol in) any fluid with proper density followed by a brief centrifuging period was sufficient to concentrate a purified biological fraction at the meniscus. For smaller microorganisms, a dual-fluid system proved to be better. This involved adding a small quantity of low-density liquid over the flotation fluid either before or after centrifugation. The microorganisms tend to concentrate in the low-density layer. One of the most significant aspects of this work involved the investigation of two groups of promising high-density fluids, the fluorochemicals and the polymer oils (chlorotrifluoroethylene). We concluded that these fluids could be applied to the problem of biological purification in a Martian environment more readily than the other fluids tested.

Another interesting facet of this work was the determination of the minimum quantity of flotation fluid which could be used to accomplish satisfactory flotation. It was established that 3 ml of fluorochemical could be used to process 1 g of soil, and that much of the microbiological material could be extracted with as little as 0.01 ml H<sub>2</sub>O. Knowledge of this type is necessary before development begins on a miniature apparatus for remote processing of a sample.

Other investigations were conducted into the possibility of treating a collected soil sample to release the microflora which are sometimes tightly attached to the inorganic particles. Essentially, two basic treatments were employed: ultrasonic vibration, and detergents. It was possible to increase the viable count in the treated samples by a combination of these techniques. But the increased yields do not appear to be worth the additional expenditure of energy in a power-limited Martian probe. Furthermore, some trials showed that these treatments must be carefully controlled to prevent the occurrence of germicidal effects.

#### III. RECOMMENDATIONS

The following outline summarizes a number of tasks which are recommended for inclusion in any subsequent program of pneumatic collection and/or centrifugal purification and concentration.

1) Sample Transfer

Build apparatus for removing the collected specimen from the sampling unit and for transferring it to a processing or evaluation device.

- 2) Sample Collector
  - a) Modify the sampler for and test it at Martian air density and temperature and at Earth gravity.
  - b) Investigate the sample losses in the system, especially at low pressure.
  - c) Consider desirability of two-stage axial-flow blower for higher pressure ratio at low air density and a higher over-all efficiency.
  - d) Alter the design to allow more satisfactory packaging and ejection of the aerosolizer unit.
  - e) Improve aerosolizer mobility by redesigning the track type vehicle or by designing a four-wheel pneumatic-tire model.
  - f) Test the sampler over lichen-covered rocks, sand confined in a binding matrix (i.e., frozen moisture), and other surfaces likely to be found on Mars. / Consider the desirability of having mechanical attachment for pulverizing and sampling such surfaces.
  - g) Consider sampler modifications and compromises for a one operating cycle system versus a many operating cycle system.
- 3) Density-Gradient Studies
  - a) Make a literature search to determine what work has been done to establish a correlation between the density gradient and organic content of soils.

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b) Conduct a laboratory investigation directed toward developing a simple procedure for automatically determining the density gradient of a soil with sufficient resolution to assure a reliable correlation between density gradient and organic content.

#### 4) Flotation

- a) Conduct a quantitative evaluation of the value of purification and concentration by flotation, for use in microscopic, enzymatic, and growth experiments.
- b) Continue present series of flotation experiments but confine them to the more promising fluids and techniques established to date.
- c) Build a miniature centrifuge, to be used with either the sample-transfer device or the sample collector for both density-gradient studies and purification-concentration work.

# APPENDIX A DEVELOPMENT OF THE SAMPLING UNIT

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#### APPENDIX A

#### DEVELOPMENT OF THE SAMPLING UNIT

#### I. INTRODUCTION

This appendix contains a detailed description of the effort that was devoted to development of the sampling unit. Data are presented in a generally chronological order as a means of illustrating the approach taken. A majority of this appendix has been prepared by compiling information previously presented in monthly progress reports.

#### II. PRELIMINARY CALCULATIONS

#### A. Basic Assumptions

Before designing a sample-collection system, we felt that we would have to have some knowledge regarding sample size, performance characteristics of the system's components, and magnitude of losses throughout the system. Because some trade-off is usually possible between these parameters, it was desirable to generate data of a general nature which could be evaluated collectively to determine the best combination of sample size versus size, weight, power consumption, and reliability of the sampling unit. Our preliminary calculations were therefore devoted to determination of the flow and pressure-drop characteristics of the collection system and to establishment of basic design parameters. (Blower-performance checks are reported later in Appendix A; particle-size experiments are presented in Appendix B.) We had determined during our feasibility study of the problem that collection could best be accomplished by aerosolizing surface materials with a highvelocity air stream and collecting the particles thus generated on impaction plates. We began our development program by reviewing the various factors involved in this process.

## B. Transport of Particles

## 1. Air Velocity

A basic requirement for transporting a particle upward in a stream of air is that the particle's drag force be greater than the gravitational force. The drag force can be expressed by

$$F_{D} = \frac{3\pi\mu D_{p}V}{C} \tag{A-1}$$

where  $F_D = drag$ 

 $\mu = air viscosity$ 

D<sub>p</sub> = particle diameter

V = air-stream velocity

C = Cunningham slip correction factor (can be assumed equal to 1 for particles larger than 50 microns).

The gravitational force exerted on a spherical particle can be expressed by

$$F_g = mg = \frac{\pi}{6} \rho_p D_p^3 g$$
 (A-2)

where  $F_g = gravitational force$ 

ρ = density of particle (assumed much larger than air density)

g = acceleration due to gravity.

Equating gravitational force and drag force to find the minimum upward air velocity we have

$$\frac{3\pi\mu D_{\mathbf{p}}V}{C} = \frac{\pi}{6} \rho_{\mathbf{p}}D_{\mathbf{p}}^{3}g.$$

By transposing and solving for velocity we obtain

$$V = \frac{\rho D_{p}^{2} g C}{18 \mu}.$$
 (A-3)

Computations were made at particle diameters of 50, 100, 200, 300, and 400 microns and the following values for Earth conditions:

$$\rho_{\rm p} = 1.2 \, \text{g per cm}^3$$
 $g = 980 \, \text{cm per sec}^2$ 
 $C = 1$ 
 $\mu = 1.4 \times 10^{-4} \, \text{dyne-sec per cm}^2$ 

A sample velocity calculation for Earth ( $V_{\rm E}$ ) was

$$V_E = \frac{1.2 \times (50 \times 10^{-4})^2 \times 980 \times 1}{18 \times 1.4 \times 10^{-4}}$$

$$V_{\mathbf{E}}$$
 = 11.7 cm per sec = 23 fpm.

Similar computations were made for Mars conditions by substituting these values:

$$\rho_{p} = 1.2 \text{ g per cm}^{3}$$

$$g = 380 \text{ cm per sec}^{2}$$

$$C = 1$$

$$\mu = 1.6 \times 10^{-4} \text{ dyne-sec per cm}^{2}.$$

A sample velocity calculation for Mars ( $V_{M}$ ) was

$$V_{M} = \frac{1.2 \times (50 \times 10^{-4})^{2} \times 380 \times 1}{18 \times 1.6 \times 10^{-4}}$$

$$V_{\mathbf{M}}$$
 = 3.96 cm per sec = 7.8 fpm.

The results can be tabulated as follows:

	Velc (fp	ocity m)
Particle Size (microns)	Earth - V <sub>E</sub>	Mars - V <sub>M</sub>
50	23	7.8
100	92	31
200	368	125
300	827	280
400	1470	498

By examining the equation for velocity, it is possible to deduce the effect on particle size transported of varying particle density at a fixed air velocity. A decrease in density by a given ratio will result in an increase in particle size transported by the square root of the given ratio.

## 2. Tube Sizes at Various Flow Rates

The expression for calculating the tube diameter D<sub>t</sub> is

$$D_{t} = 12\sqrt{\frac{4Q}{\pi V}}$$

where  $D_t = diameter of tube (inches)$ 

Q = air flow rate (cfm)

V = air velocity (fpm).

This equation is readily obtained from the basic relationship that flow rate equals velocity times cross-sectional area, assuming a noncompressible fluid. The assumption is quite valid in this case because pressure drops are very small in relation to total atmospheric pressure.

Tube diameters were calculated for flow rates of 5, 10, and 20 cfm and for all of the velocities tabulated previously. These results are presented graphically in Figure A-1.

#### C. Impaction

The technique of collecting a sample of particles larger than a given particle-size cutoff has been discussed in previous reports. The effort reported here is the determination of the relationship between particle-size cutoff, nozzle diameter, flow rate, and air velocity through the nozzle for both Earth and Mars environments.

#### 1. Nozzle Diameter

The "inertial parameter"  $\psi$  for a circular nozzle impactor is defined by the expression

$$\Psi = \frac{\rho_p \text{CVD}_p^2}{18\mu D_n} \tag{A-4}$$

where V = velocity of air in nozzle

D<sub>p</sub> = particle diameter

 $\mu$  = absolute viscosity of air

 $D_n = nozzle diameter.$ 

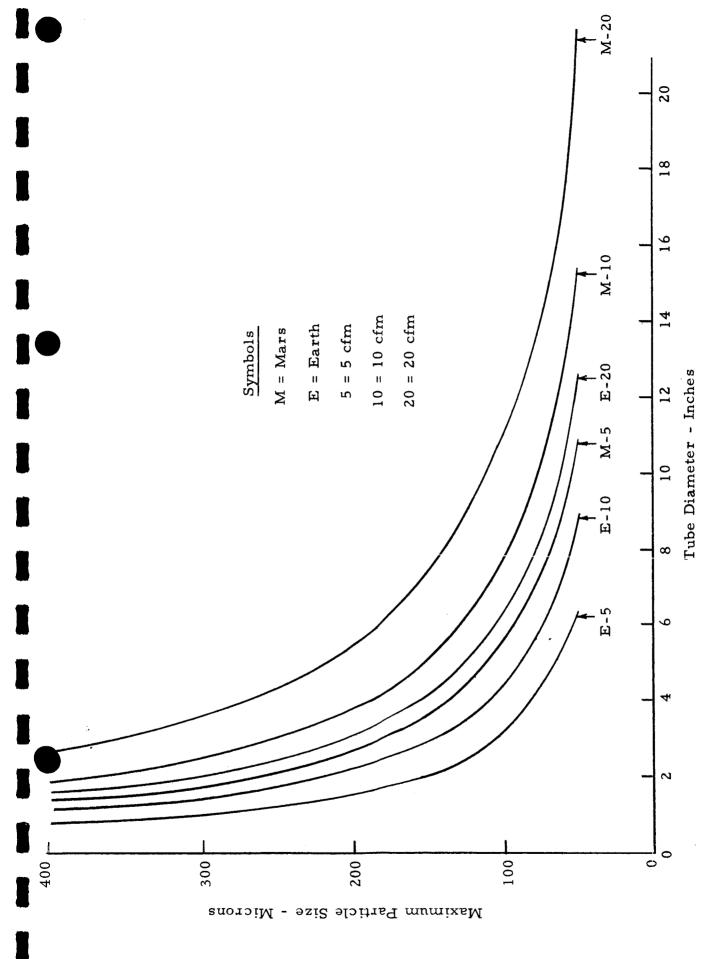


Figure A-1, Maximum Particle Size Transportable versus Tube Diameter

The relationship between the inertia parameter and the collection efficiency (percent) has been shown in previous reports. A conservative value for the inertial parameter (which will yield 50 percent collection efficiency) is

$$\psi = (.36)^2 = .129.$$

Using the relationship Q = VA and solving for V, the following can be written:

$$V = \frac{4Q}{\pi D_n^2} \tag{A-5}$$

where Q = flow rate.

Substituting the value for V in the previous equation gives

$$\Psi = \frac{\rho C4QD_p^2}{18\mu D_n \pi D_n^2}.$$

And solving further for  $D_n$  we have:

$$D_{n} = \left[\frac{2 \rho CQD_{p}^{2}}{9 \mu \pi \psi}\right]^{1/3}.$$

Values were computed for nozzle diameters under both Earth and Mars conditions at particle diameters of 2.5, 5, and 10 microns and at flow rates of 5, 10, and 20 cfm. Substituting the following values and making a sample calculation,

$$\rho_p = 1.2 \text{ g per cm}^2$$

C = 1.0 (Earth)

C = 1.11 - 1.44 (Mars) (a function of particle size)

$$Q = 10 \text{ cfm} = 4.72 \times 10^3 \text{ cm}^3 \text{ per sec}$$

$$D_{\rm p} = 10 \text{ microns} = 10^{-3} \text{ cm}$$

$$\mu = 1.4 \times 10^{-4}$$
 dyne-sec per cm<sup>2</sup> (Earth)

$$\mu = 1.6 \times 10^{-4}$$
 dyne-sec per cm<sup>2</sup> (Mars)

$$\Psi = .129.$$

$$D_{n} = \left[ \frac{2 \times 1.2 \times 1.0 \times 4.72 \times 10^{3} \times (10^{-3})^{2}}{9 \times 1.4 \times 10^{-4} \times \pi \times .129} \right]^{1/3}$$

 $D_n = 2.81$  cm (10-micron cutoff, 10 cfm, on Earth)

The results of all of the nozzle-diameter calculations are shown in graphic form in Figure A-2.

## 2. Nozzle Velocity

Knowing the flow rate and nozzle diameter, it is quite simple to compute the nozzle velocity using the aforementioned expression

$$V = \frac{4Q}{\pi D_n^2} .$$

Velocity is used in a later section of this report for computing pressure drops. Without going into any further detail, the results can be tabulated as shown in Table A-I.

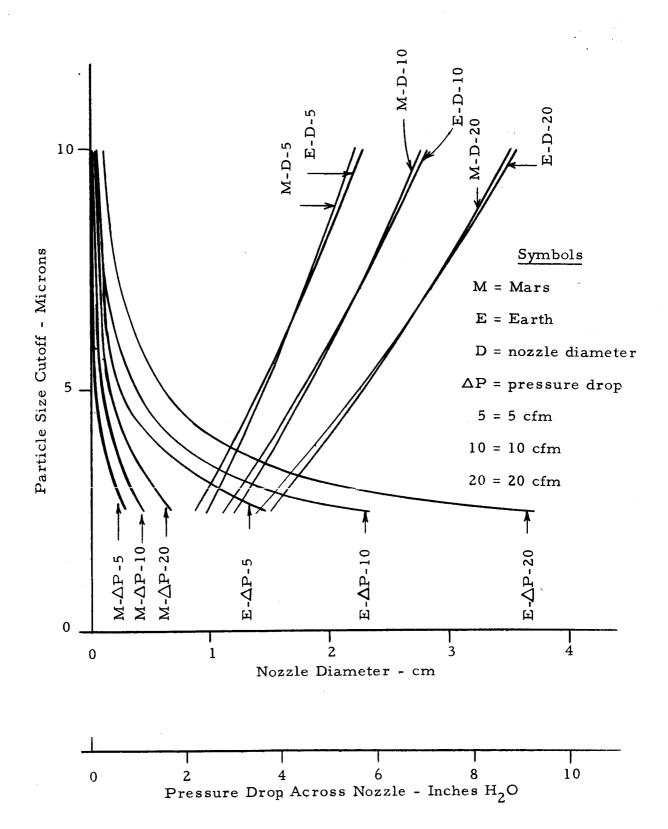


Figure A-2. Impactor Nozzle Diameter versus Particle Size Cutoff at 50 Percent Collection Efficiency

Table A-I. Nozzle Velocity at Varying Flow Rates and Particle-Size Cutoffs

Planet	Flow Rate (Q) (cfm)	Particle Cutoff (microns)	Nozzle Velocity (fps)
Earth	5	2. 5	126
Earth	5	5	50
Earth	5	10	19
Earth	10	2.5	160
Earth	10	5	63
Earth	10	10	25
Earth	20	2.5	201
Earth	20	5	79
Earth	20	10	31
Mars	5	2.5	108
Mars	5	5	48
Mars	5	10	20
Mars	10	2.5	137
Mars	10	5	60
Mars	10	10	25
Mars	20	2.5	171
Mars	20	5	76
Mars	20	10	32

#### D. Pressure Drops

Typically, a blower will develop a specific pressure head at a particular flow rate when connected to some fixed air-flow system. Such curves of pressure versus flow rate are generally included in the literature published by blower manufacturers concerning their blowers.

The pressure head developed by a blower must be equal to the resistance or total pressure drop of the air flow system to which it is connected. In general, an air flow system will have an increasing total pressure drop with increased flow rate, whereas a blower will have a decreasing pressure head with increased flow rate. Therefore, if total pressure drop and pressure head curves are plotted on the same graph, their point of intersection will yield both the flow rate at which the system will operate and the associated pressure head.

Figure A-3 is a schematic representation of the pneumatic collection system as it was originally envisioned. This consists of a transport tube, impactor, blower, exhaust tube, and aerosolizing device. The total pressure drops computed herein are based on this configuration. The final design varied only slightly in concept from that shown.

## 1. Transport Tube

This tube was assumed to be 10 ft long for purposes of pressure-drop calculations. Samples of "Flexflyte" light weight flexible ducting were procured from the Flexible Tubing Corporation, Guilford, Connecticut. Pressure drops for this tubing are based on the manufacturer's published air-friction chart.

This tubing has a construction of one-ply, Neoprene-coated Fiberglas fabric over galvanized spring-steel wire helix, cemented and bound together with coated Fiberglas cord.

Pressure drops for the transport tube determined from the manufacturer's air-friction chart for the various combinations of flow rate and maximum particle size transportable are tabulated for Earth in Table A-II. Tube sizes for these combinations are shown in Figure A-1.

The exhaust tube was assumed to be of a larger area so that the pressure drop in it would be approximately one-half of that in the transport tube.

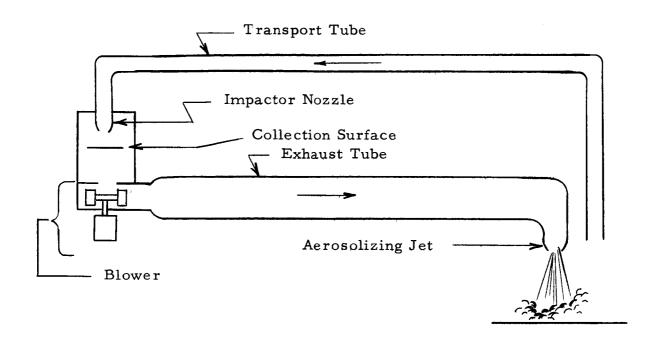


Figure A-3. Pneumatic Particle Collection System
Considered for Pressure Drop Calculations

Table A-II. Transport Tube Pressure Drop, Earth Conditions

Maximum Particle Size (microns)	Flow Rate (cfm)	ΔP-Transport Tube Inches H <sub>2</sub> O(Earth)
100	r-	0.01
100	5	0.01
100	10	0.01
100	20	0.01
200	5	0.03
200	10	0.02
200	20	0.01
300	5	0.15
300	10	0.12
300	20	0.10
400	5	0.40
400	10	0.35
400	20	0.30

## 2. Nozzle

It is assumed that the pressure drop across the nozzle only (not including the collection surface) is equivalent to the velocity pressure head of the air stream passing through the nozzle. This pressure drop can be expressed by the equation

$$\Delta P = \frac{P_1 V^2}{2gRT_1} \tag{A-6}$$

where  $\Delta P = pressure drop$ 

P<sub>1</sub> = ambient air pressure

V = air velocity through nozzle

R = gas constant

T<sub>1</sub> = ambient air absolute temperature.

The values to be substituted (for Earth and Mars respectively) and a sample calculation are as follows:

 $P_1$  = 2116 and 180 lb per sq ft

R = 53.3 and 50 (approx. for air)

 $T_1 = 520 \text{ and } 510 \text{ R}$ 

g = 32.2 and 12.4 ft per sec<sup>2</sup>

V = 25 fps (at 10-micron cutoff and 10 cfm on Earth for sample calculation)

$$\Delta P = \frac{2116 \times (25)^2}{2 \times 32.2 \times 53.3 \times 520}$$

 $\Delta P = 0.74$  lb per sq ft = 0.14 inch H<sub>2</sub>O

The results of all the calculations for a pressure drop across the nozzle are shown graphically in Figure A-2.

## 3. Total System (Earth Conditions)

Estimates were made for the pressure drop across other components of the air-flow system. In most cases, these estimates were based on a relationship with known values.

The total pressure drops (at various combinations of parameters) of the system were obtained simply by adding the computed values for the transport tube and nozzle to the total of the estimates for the various other components. All of these total pressures were plotted on a graph of pressure versus flow rate, and lines were drawn through points representing the same particle-size collection range. Thus, sixteen lines were drawn for all combinations of minima of 2.5, 4, 5, and 10 microns and maxima of 100, 200, 300, and 400 microns. These results are shown in Figure A-4.

## E. Comparison of System Pressure Drops with Blower Pressure Capability (Earth)

The axes of Figure A-4 were plotted to the same scale as two blower-performance curves obtained earlier and reproduced here for comparison (Figures A-5 and A-6). This allows Figure A-4 to be superimposed on the blower performance curves to determine the intersection points of the pressure curves.

Upon doing this, it can be seen that it is impractical to attempt the collection of particles as small as 2.5 microns with the Globe blower, since the pressure drop is substantially higher than the pressure head. The intersection occurs at a low flow rate for the Benson blower--that is, less than 5 cfm. The practical minimum-size particle collection for either blower is approximately 4 microns.

Computations were also made to determine the total pressure drop under Martian conditions for a pneumatic collection system with the general configuration shown in Figure A-3. The values for frictional losses in ducting, reported in Section II-D-1 were used. Figure A-7 is the summary of this work. Also plotted on Figure A-7 are the pressure-drop curves for the Globe Torrington and D & B blowers operating at 0.1 atmosphere. Intersection points show the capability of these blowers in terms of particle-size range that can be collected on Mars and the resulting volume flow rate. The practical minimum collected particle size is 4 to 5 microns.

At 4 or 5 micron particle-size minimums, almost any of the maximums shown could be chosen because they all intersect at reasonable flow rates. However, other conditions involving design and size must be taken into account when selecting the operating range.

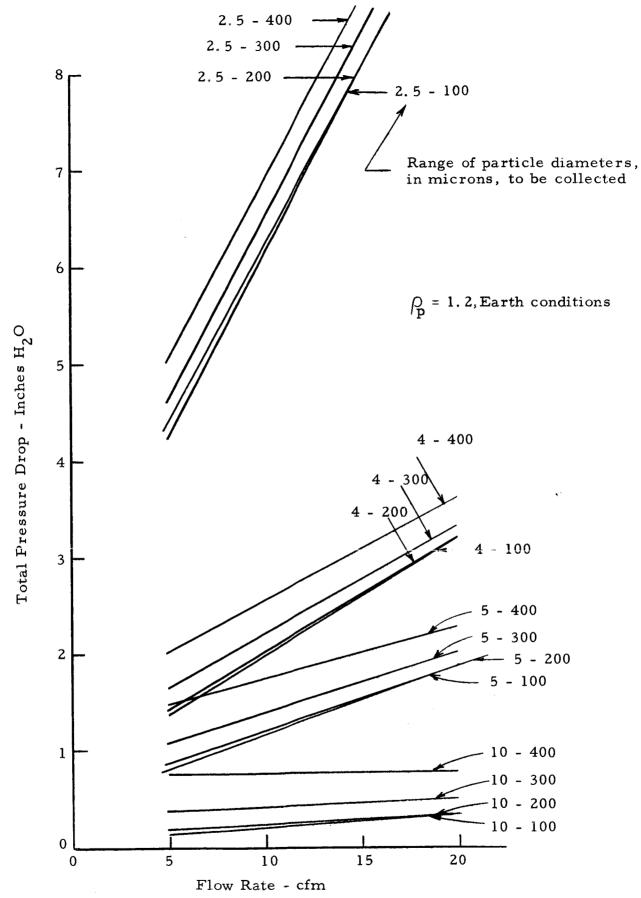
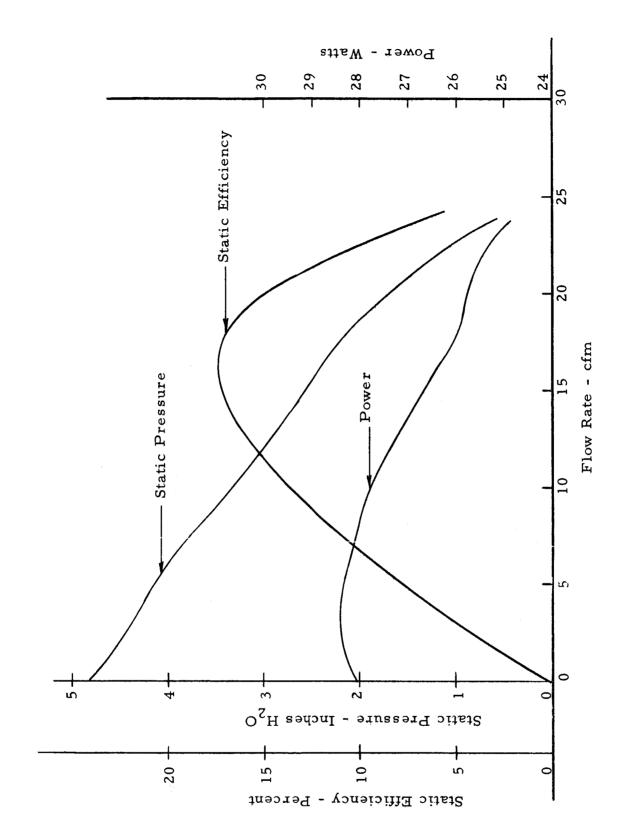


Figure A-4. Total Pressure Drop of Collection System versus Flow Rate (for Earth Atmosphere)



Performance Curves - Benson Blower (Model HP 2-2) Figure A-5.

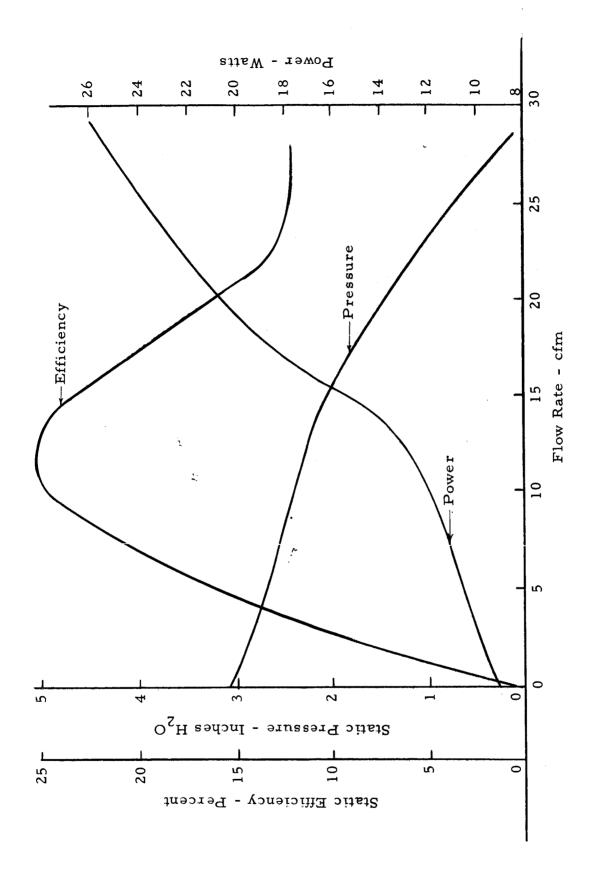


Figure A-6. Performance Curves - Globe Blower (Model No. 2B-3A-851)

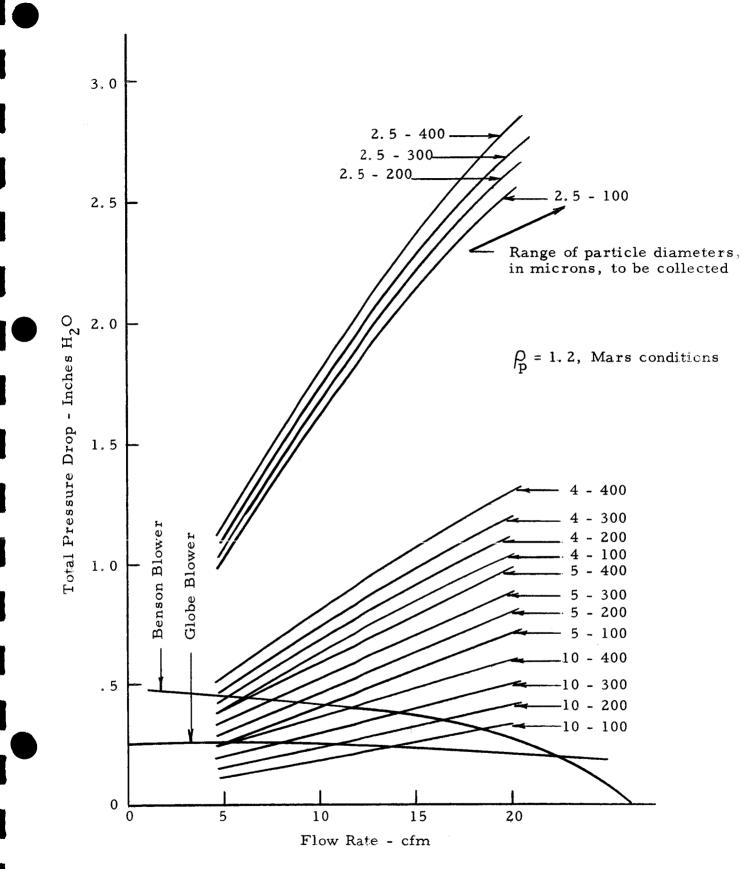


Figure A-7. Total Pressure Drop of Collection System versus Flow Rate (for Martian Atmosphere)

Some typical values for a 5 to 400 micron diameter particle-size collection range at both Earth and Martian conditions are these:

Earth Conditions	Mars Conditions		
Flow rate = 15 cfm	Flow rate = 6 cfm		
Blower = Globe	Blower = Benson		
Pressure head = 2 inches H <sub>2</sub> O	Pressure head = $0.45$ inch $H_2O$		
Transport tube diam. = 1.3 inch	Transport tube diam. = 1.3 inch		
Effluent tube diam. = 2.3 inches	Effluent tube diam. = 2.3 inches		
Nozzle diam. = 2.0 cm	Nozzle diam. = 1.4 cm		

The last two values were obtained by referring back to Figures A-1 and A-2.

#### TIL PRELIMINARY TESTING OF COMPONENTS

In the early stage of this program it was established that a motor, blower, and gearhead motor would be required. Later, as the design began to formalize, transport tubing, latching mechanisms, etc., were required. Performance testing of this equipment is reported in the following paragraphs.

#### A. Blowers

Flow rates, power consumption, and pressure drops were measured at 1/10 atmospheric pressure (equivalent to Martian air density) for a Globe model 2B-A3-851 d-c blower and a Benson model HP 2-2 a-c blower. Performance curves for these blowers (at sea-level conditions) have been reported previously in GMI Report No. 2326 and are reproduced as Figures A-5 and A-6 of this report.

The blower under test was placed in a vacuum "tunnel" or chamber with the blower inlet connected to the outside through a throttling valve and flowmeter. The pumping and throttling rates were balanced to stabilize the chamber pressure at 1/10 atmospheric pressure, and readings were taken. This process was repeated at several flow rates and at the no-flow condition. The data collected are tabulated in Table A-III, and the results are shown in Figure A-8.

These curves can be used with pressure-drop curves of the Martian collection system to determine operating characteristics of the system at 0.1 atmosphere. It is significant to note that the efficiencies of these units designed for sea-level conditions fall off markedly with decrease in air density. This is a direct result of using a motor which is overpowered at these conditions.

### B. The Martian Blower Test

A Benson motor-blower unit was modified to improve its over-all efficiency by replacing the unit's Hertner motor with a low-power Globe Model No. 53A105-1 motor. This motor draws 5 watts under load and can operate from a 115-volt, single-phase, 400-cycle power supply. Our previous tests on the Hertner/Benson unit (reported in Paragraph III-A) had shown a power consumption of 12 watts in a Martian-equivalent air density.

The modified Benson blower was tested in a vacuum chamber at 1/10 atmospheric pressure. The blower's designed speed of 22,000 rpm was verified using a Strobatac. Flow rates, power consumption, and pressure rise were measured and recorded at several flow rates and at a no-flow condition. These data are presented in Table A-IV, and the results shown in Figure A-9. Use of a motor sized precisely for this application resulted in very good performance and greatly improved efficiency. Some of the difference in shape between the static pressure curve for this unit and that for the Hertner motor-powered blower recorded in Figure A-8 can be attributed to an air-flow reversing section at the blower exit; the earlier test unit did not have this feature.

Table A-"II. Data from Performance Test of Benson Blower at Low Atmospheric Pressure

Flow Rate (cfm)	Pressure (inches H <sub>2</sub> O)	Power Output (watts)	Ambient Pressure (mb)	Efficiency (%)	Power Input (watts)
0	0.580	0	100	0	11.4
3.87	0.468	0. 213	101	1.75	12.2
10.27	0.419	0.505	99	4.00	12.6
14. 1	0.384	0.636	99. 5	5. 05	12.6
20.3	0. 269	0.642	100	5. 65	11.4
23.5	0.170	0.470	100.7	4. 27	11.0
27. 3	negative reading		100		10.6
0	4.88	0	988	0	25.6

Table A-IV. Data for Globe Motor in Benson Blower

Flow Rate (cfm)	Pressure (inches oil Sp.G. = 0.935)	Power Input (watts)	Ambient Pressure (mb)	Power Output (watts)	Efficiency (%)
0	0. 527	4.9	101	0	0
2.19	0.452	4.9	99.5	0.109	2. 22
3. 24	0.417	4.9	99. 0	0.135	2.76
16.3	0. 322	5.2	100.5	0.578	11.0
22. 2	0.171	4.9	100.0	0.417	8.0
14.41	0, 345	5.2	100.0	0.547	10.5
10.12	0.375	5.2	100.0	0.417	8.00
7.45	0.393	5.2	100.0	0.322	6. 20
5.72	0.407	5.2	100.0	0.255	4. 9
4.72	0. 415	5. <b>2</b>	100.0	0.217	4.17

- (1) Benson 200-volt, 400-cycle, vane-axial blower
- (2) Globe 27-volt d-c centrifugal blower

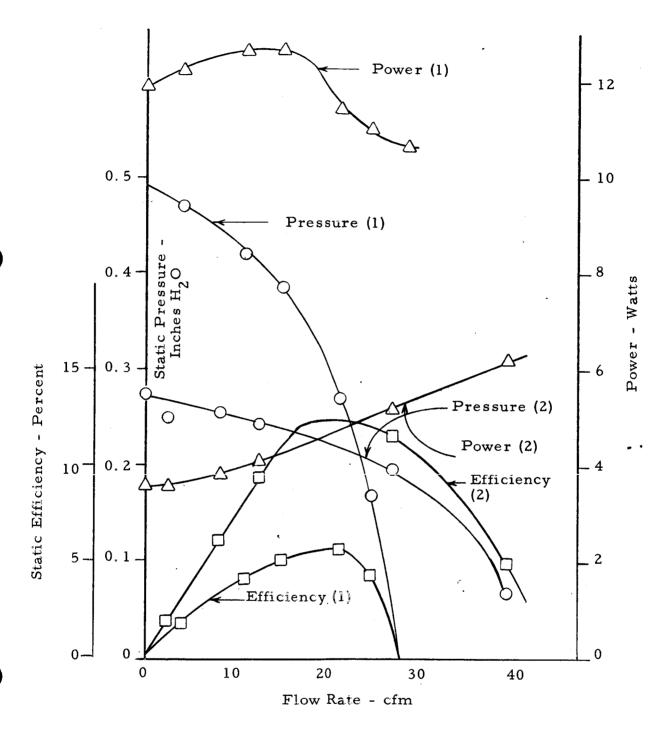


Figure A-8. Performance Curves for Two Blowers at a Martian Equivalent Air Density of 0.0075 lb/ft<sup>3</sup>

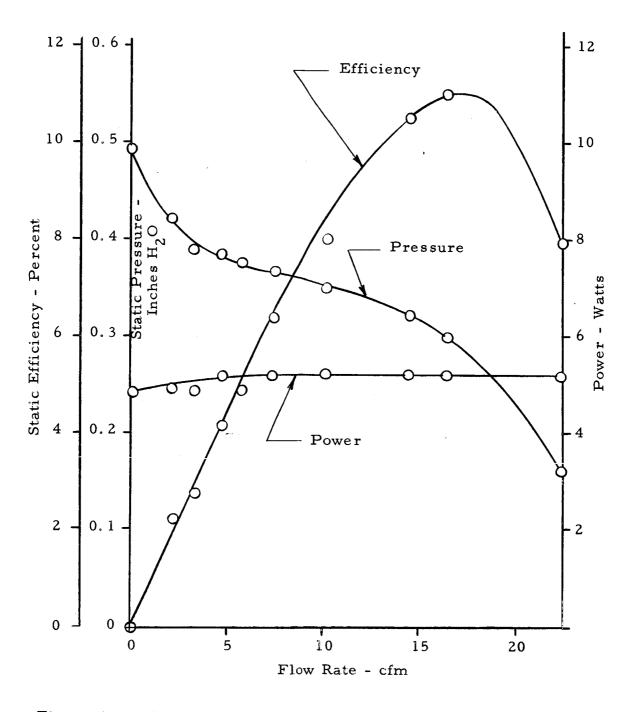


Figure A-9. Performance Curves for Benson Blower with Globe No. 53A105-1 Motor at a Martian Equivalent Air Density of 0.0075 lb/ft<sup>3</sup>

### C. Globe A-C Axial Blower

In the interest of evaluating as many small blowers as possible, several units were purchased (some surplus items at low cost) for examination and testing. One of these was the Globe type SC (Part No. 19A-650-1) axial blower. Because this type of blower develops a low differential pressure (according to manufacturer's literature), testing was limited to determination of power consumption and speed with no attempt at measuring air flow or back pressure.

This unit was designed to run on 400-cycle, 115-volt, single-phase (with capacitor) or three-phase power, and is rated at 30 cfm maximum or 0.8 inches  $\rm H_2O$  maximum.

Operating the unit on single-phase power (using a 0.25-microfarad capacitor), the speed was measured at 10,800 rpm and power consumption at 8.5 watts.

Operating the unit on three-phase power, the speed was 10,600 rpm and total power consumption was 11.1 watts. We noted that the wattage decreased slightly when the air flow was blocked.

#### D. A Redesigned Centrifugal Blower

A Torrington Model No. 2-1/2 centrifugal blower was modified to redirect the effluent air. Its purpose was to make the blower system much more compact than the usual scroll design. A new housing was designed and fabricated to fit the old blower wheel and motor and to cause the redirected flow of air. This housing was made to mate with the rest of the sampler and had an impactor stage incorporated in it. Details are shown in Figure A-10.

This blower was tested at Earth conditions, and measurements were made of flow rate, pressure drop, and power consumption. Voltage input was held constant. These data are tabulated in Table A-V and plotted in Figure A-11. This blower wheel had the same dimensions and was of the same design as

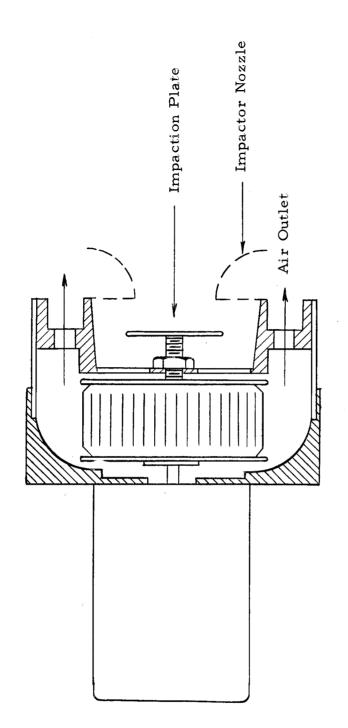


Figure A-10, Redesigned Centrifugal Blower

No.

Table A-V. Data from Performance Tests on "Redirected Flow" Centrifugal Blower

Flow Rate (cfm)	Pressure (inches H <sub>2</sub> O)	Power Input (watts)	Efficiency (%)	Power Output (watts)
13.3	0.2	14.5	2. 1	0.31
12.7	0.3	14.0	3. <b>2</b>	0.45
11.5	0.45	13.7	4.5	0.61
8.8	0.7	13.2	5.5	0.72
7.0	0, 9	12.6	5. 9	0.74
5.7	1.05	12.3	5.7	0.70
3.0	1.35	11.5	4.2	0.48
0	1.7	11.2	0	0

the blower wheel in the Globe blower (Model No. 2B-3A-851) tested previously. Since the speed was the same, the performance curves for the "redirected flow" centrifugal blower can be compared with the performance curves shown in Figure A-7. This comparison shows that the redesigned centrifugal blower developed considerably less pressure, with less air flow, resulting in a much lower efficiency.

### E. Haydon Printed-Circuit Motor

A rather recent development of the Haydon Instrument Company (Waterbury, Connecticut) is a compact printed-circuit "Series 9100" d-c motor. Because this motor possessed a combination of small size, light weight, high speed, and low power consumption, and because it may possibly have several applications (with or without available gear trains) on the Mars Microscope System, we decided to purchase one unit (without gear train) for test and evaluation.

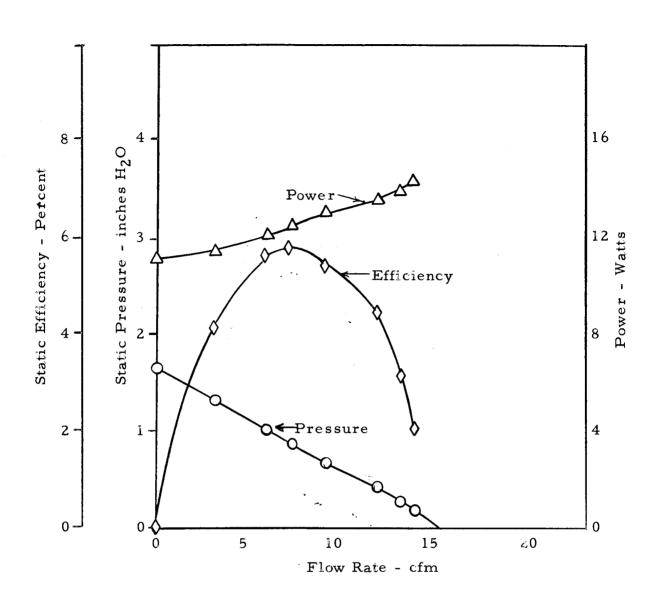


Figure A-11. Performance Curves for Redesigned Centrifugal Blower at a Standard Air Density of 0.075 lb/ft<sup>3</sup>

The motor was connected to a variable d-c power supply and operated at a no-load condition. Readings of voltage applied were made with a Triplett Model 630NA volt-ohmmeter, amperage with a Simpson Model 260 VOM, and speed with a General Radio Model 1531A Strobatac. These recorded data and computed values of power consumption in watts are presented in Table A-VI, and in graphical form in Figure A-12.

The measured values agreed closely with the manufacturer's data. The nominal rating of this motor is 1.5 v, 0.25 a, 10,000 rpm, and weight (without gear train) of 2-1/2 oz. A motor of this type (with gear train) was used for driving an aerosolizer extraction mechanism described later in this report.

## F. Gaylord Reeves Motor

A 400-cycle, 26-volt, single-phase (with capacitor) motor was purchased from Gaylord Reeves for testing and evaluation because of the high efficiency claim made for this motor. A transformer was used to reduce the output voltage of the Behlman ''Invertron'' 400-cycle power-supply voltage to the required 26-volt level.

After a short operating time the motor became very hot and had to be shut off. During this time, it was noted that power consumption was extremely high at 75 watts, and that the speed was over 22,000 rpm. This motor was considered unsuitable for further use on this project.

## G. Daystrom Size 8 Motor

Another motor purchased because of its very small size for possible use in driving the wheels on the aerosolizer was a Daystrom Size 8 motor. This again is a 400-cycle, 26-volt, two-phase (single-phase with capacitor) type of motor, and was connected to the Behlman power supply utilizing the transformer.

Table A-VI. Performance Data on Haydon Series 9100 Printed Circuit Motor

Voltage (v)	Current (ma)	Speed (rpm)	Power Consumption(watts)
0. 72	135	4,800	0.097
0.91	158	6,000	0.144
1.09	182	7,200	0.198
1.18	196	7,700	0.231
1.43	220	9,350	0.315
1.75	272	11,250	0.476
2.07	330	13,200	0.683
2.50	410	15,400+	1.025
2.75	465	17,500	1.28
3.1	670	22,200	2.08
3.7	820	25,900	3.03
5.0	1,180	33,540	5. 90

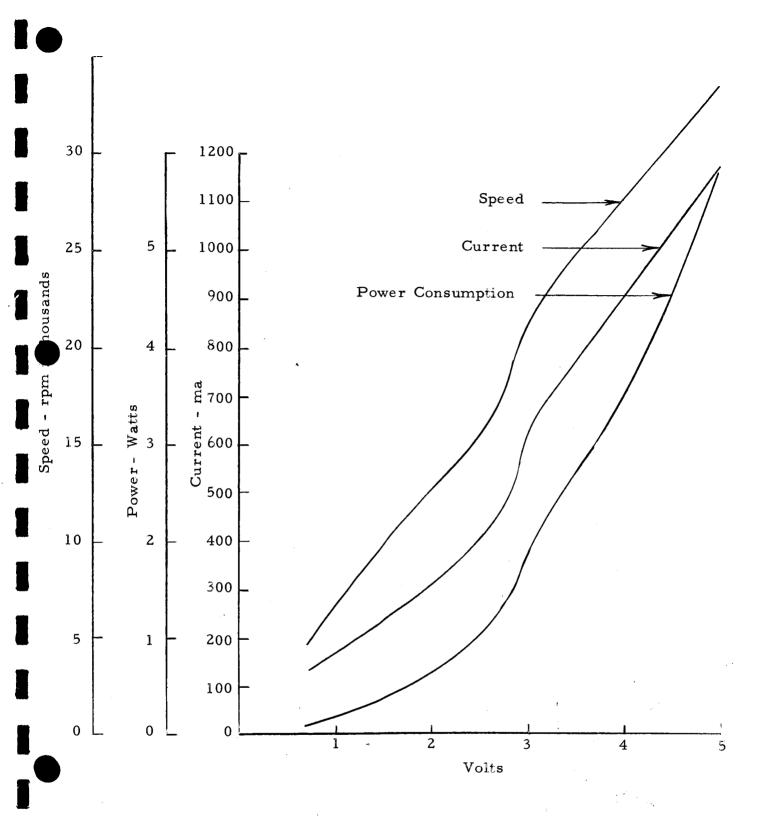


Figure A-12. Performance Curves for Haydon Series 9100 Printed Circuit Motor

The motor ran at a speed of approximately 5,400 rpm and drew a power of 2.5 watts. This power did not vary noticeably with the application of load to the motor output shaft.

A speed reducer was purchased for this motor to achieve an output speed of approximately 6 rpm. This combination was then eventually used to drive the wheels of the aerosolizer unit.

#### H. Miniature D-C Gearhead Motor

A gearhead d-c motor (7/8 inch diam. x 2-5/8 inch long) was purchased for evaluation for possible use in driving the wheels of the aerosolizer. This motor, made by American Electronics, Inc. and similar to ones made by Globe Industries, was designed for a nominal voltage of 27.5 v and an output speed of 25 to 30 rpm.

The motor was tested at various voltages, and measurements were made of speed and current consumption, both with and without the gearhead attached. This provided an interesting comparison, which showed the power lost through the gearhead. This power loss amounted to only 130 to 150 milliwatts at nominal voltage, which represents approximately 8 percent of motor input power.

The speed of the motor alone was observed at 12,000 to 14,000 rpm at nominal voltage, and we can thus estimate the gear ratio at approximately 500 to 1.

The recorded data (and computed power values) are shown in Table A-VII. Curves showing input current, power, and output speed with and without the gearhead are shown in Figure A-13. Also shown are curves of power loss in the gearhead and efficiency of the gearhead relative to input power.

## I. Pressure Drop in Flexible Ducting

Measurements were made of the pressure drop across a 10-ft length of various sized (1.0 inch, 1.5 inch, 2.0 inch) Flexflyte flexible ducting at various

Table A-VII. Performance Data of Miniature D-C Gearhead Motor (American Electronics, Inc.)

	Motor	Motor without Gearhead	arhead	Motor	Motor with Gearhead	head	Power Loss	Gearbead
Voltage Applied	Current (ma)	Speed (rpm)	Power (watts)	Current (ma)	Speed (rpm)	Power (watts)	in Gearhead (watts)	Efficiency (%)
30	61	14,800	1.83	99	30, 5	1.98	0.15	7.6
27	59	13,300	1.59	64	27.4	1.73	0.14	8.1
25	58	12,350	1.45	63	25.4	1.58	0, 13	8. 2
20	55	9,700	1.10	61	20.0	1.22	0.12	9.8
15	. 52	7,050	0.78	57	14.5	0.86	0.08	9.3
10	49	4,300	0, 49	53	8.9	0.53	0.04	7.6
9	46	2,200	0. 28	49	4.5	0.29	0.01	3.5

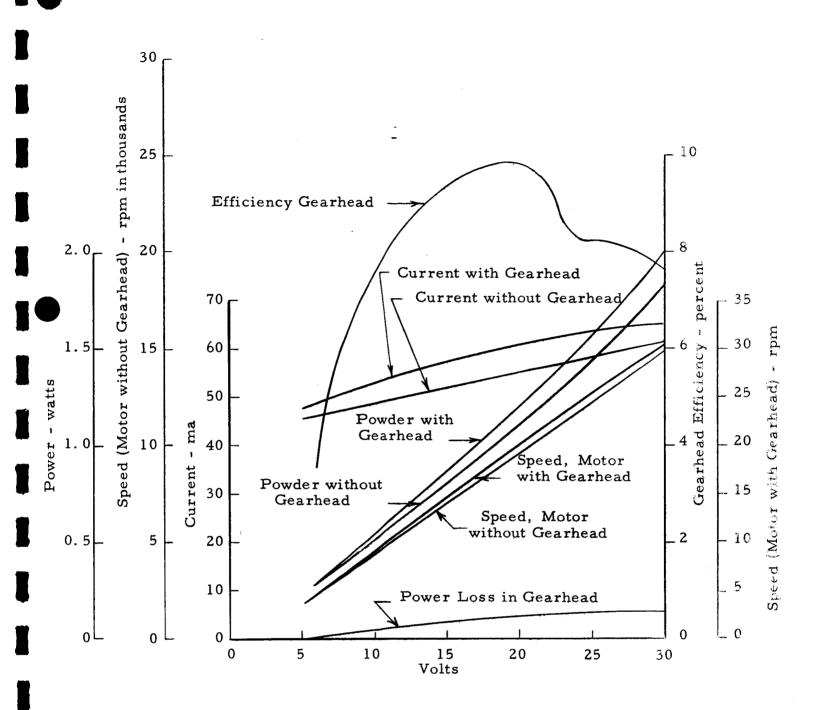


Figure A-13. Performance Curves of Miniature D-C Gearhead Motor (American Electronics, Inc.)

flow rates for Earth conditions. It was found that pressure drops were approximately twice the published values in the manufacturer's literature. The data from these tests are shown on Figure A-14 and recorded in Table A-VIII, upper half.

The Martian pressure drops were computed using the expression (from Marks, L.S. Mechanical Engineers' Handbook, 4th ed. New York, McGraw-Hill, 1951, p. 267).

$$p_1 - p_2 = 2720 \text{ f} \frac{LSQ^2}{D^5}$$

where  $p_1 - p_2 = pressure drop in psi$ 

f = friction factor

L = length of tube in ft

S = specific gravity referred to water

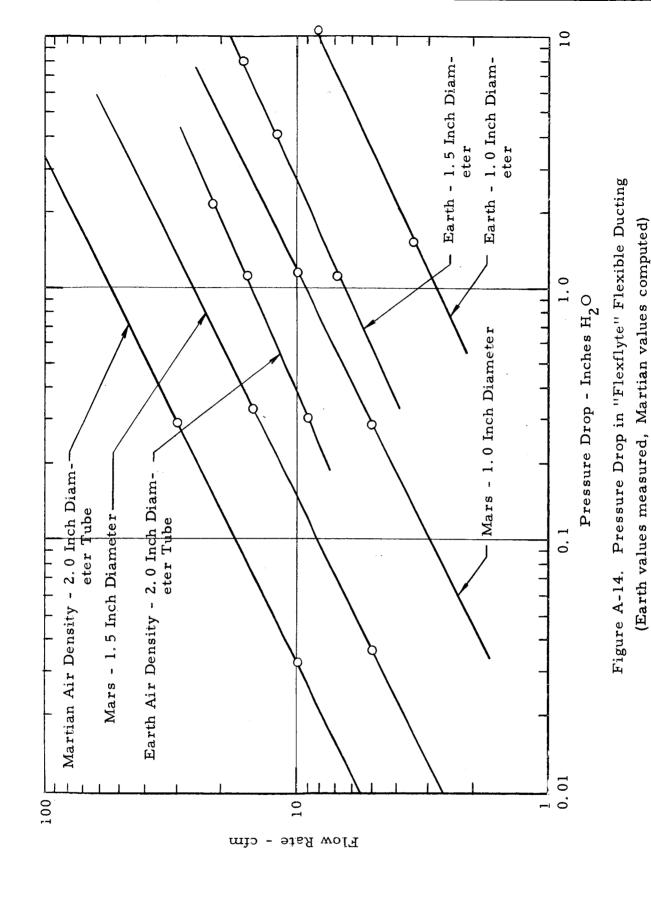
Q = flow rate in cu ft per sec

D = diameter of tube in inches.

This equation was first used to determine the friction factor at measured Earth conditions. The friction factor was then corrected by graphical means to the lower Reynolds number for Mars, and the resulting pressure drops were calculated. These calculated values are listed in Table A-VIII, lower half.

# J. Inflatable Righting Structure

During the period when the various components were being evaluated, considerable thought was given to ways of utilizing the blower effluent to orient or translate the aerosolizer-collector nozzle. It is interesting that if an inflatable structure does not leak, the effluent air can be directed into it without loss of operating efficiency to the collector. This is done by placing an orifice that is larger than the aerosolizer nozzle in the effluent air duct. When the operating cycle is initiated, the air will thus flow



A-38

Table A-VIII. Data Recorded on Measurements of Pressure Drops Across 10 Ft Lengths of "Flexflyte" Tubing, Standard Conditions

Nominal Tube Size (inches)	Flow Rate (cfm)	Pressure Drop in 10 Ft (inches H <sub>2</sub> O)
1	10.5	1.68
1	8.3	1.03
1	3.46	0. 15
1	10.4	1.63
1-1/2	16. 7	0.78
1-1/2	12. 1	0.40
1-1/2	6. 9	0.11
1-1/2	16. 5	0.78
2	21.7	0.21
2	16.0	0.11
2	9. 1	0. 03
2	21.7	0.21

# Calculated Pressure Drop in Flexflyte Tubing in Mars Atmosphere

Flow Rate	Dian	neter	$p_1 - p_2$		Calculated Friction
(cfm)	(inches)	(inches) <sup>5</sup>	psi	(inches H <sub>2</sub> O)	Factor
				_	
10	2.04	35.3	0.00116	0.032	0.045
30	2.04	35.3	0.0104	0.288	0.045
5	1.51	7.85	0.00130	0.036	0.045
15	1.51	7.85	0.0117	0.324	0.045
5	1.00	1.00	0.0102	0.282	0.045
10	1.00	1.00	0.0408	1.13	0.045

into the inflatable device until sufficient pressure is developed to force air through the aerosolizer nozzle. The inflatable will remain in this pressurized state without any additional expenditure of power.

The first device tested which employed this concept was simply a torroid placed around the aerosolizer-collector nozzle at the end of the transport tube. This torroid, when pressurized, unerringly positioned the nozzle for optimum sampling. A number of righting structures and the inflatable tires used in subsequent models resulted from these early tests.

# IV. CONSTRUCTION OF THE SAMPLING UNIT

When the preliminary calculations and some of the preliminary testing had been completed, a sampling unit was designed and built. The purpose of this unit was to test the performances of various components. In addition to the one complete sampling unit, two alternate aerosolizers were built. Figure A-15 is a sectional view of the basic system in its packaged state. The unit is 10-1/4 inches long, 3 inches in diameter, and for the most part is fabricated from aluminum.

### A. Blower

The motor-blower unit used was manufactured by Benson Manufacturing Company. It was chosen because it provided the highest static pressure for its size of any blower located, and because its size and shape permitted a clean, compact unit design. A motor that operates this blower more efficiently in a Martian environment was purchased and tested, as reported in an early section of this report. This motor was completely interchangeable with the motor supplied as standard equipment with the Benson blower.

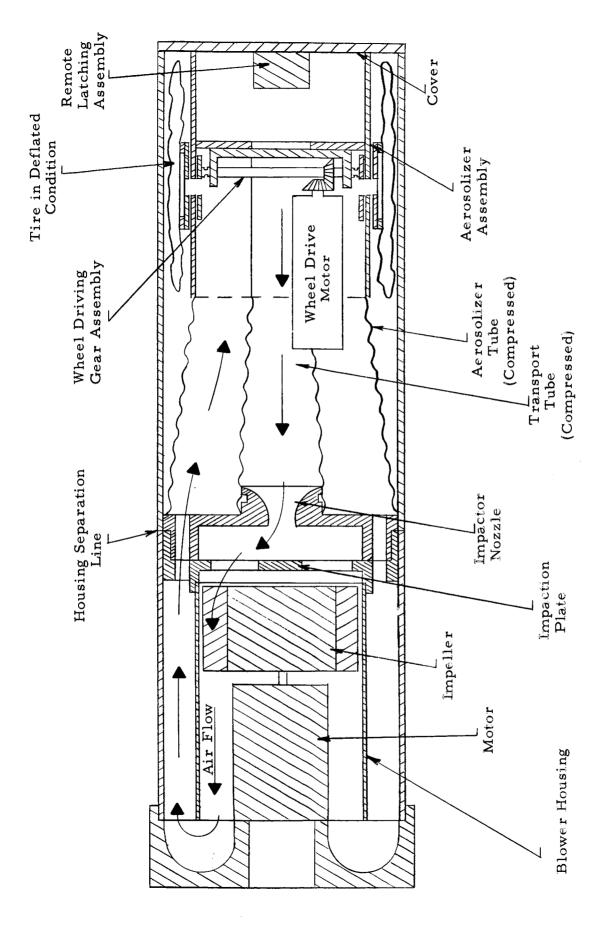


Figure A-15. Sectional View of the Basic Sample Collection Unit in its Packaged State

### B. Impactor

The impaction stage is an aluminum plate with two rows of concentric holes. The inner and outer row permitted passage of the blower influent and effluent, respectively. Provision was made for a glass slide with a specially-prepared surface to be attached to the center of this impaction plate. The impactor nozzle had a throat diameter of 2 cm and was positioned approximately 1 cm from the impaction plate. The collector housing separated at the interface of the impactor plate and nozzle, thereby providing ready access to the collected sample and making it possible to replace the impactor with some other collection device or to add a section containing a processing or sample-transferring mechanism.

## C. Housing

The general shape of the housing was suggested by the cylindrical blower and by the cylindrical packaged state of the transport tube. Three-inch diameter tubing was chosen because it was readily available and because its size was compatible with the dimensions of components selected from the analysis section.

#### D. Transport Tubes

A specially-designed, light weight, highly-flexible transport tube was built of helical spring wire coils covered with plastic film or plastic-coated fabric. A number of combinations of coverings were tried to determine the effect or desirability of their various physical properties. Consideration was given to how readily they could be packaged, how resistant they were to abrasion and pinhole formation, and how readily they could be fabricated into tubular coverings for the helical coils to form the transport tubes. Electrically conductive films were also considered as a means of removing the static charge which tended to build up on the tube and remove particles from the air stream. The major requirement was to design a system of tubes

which was 120 inches long when in use and only 2 to 3 inches long when packaged. This compression ratio is an order of magnitude greater than that attainable with commercially-available flexible ducting. The inner and outer tubes were 1.3 and 2.5 inches in diameter respectively, in keeping with the design parameters established in the preliminary calculations for collected particles in the size range of from 5 to 400 microns.

#### E. Aerosolizers

Recognizing that the collectors' performance will greatly depend on the aerosolizer, three aerosolizer units were fabricated. Each unit had some advantages over the others. For instance, the unit shown in Figure A-16 utilizes the blower effluent to inflate a righting structure and to inflate the tires to provide mobility. This utilization of the blower effluent costs nothing in additional power. Since the unit should negotiate areas of very fine dust and moderate physical barriers equally well, it was anticipated that the nozzle-to-ground spacing would remain somewhere near optimum. The inflatable tires offered maximum bearing surface with minimum package volume.

Two alternate aerosolizers were built and tested in addition to the inflatable-tire mobile particle aerosolizer. The first is shown schematically in Figure A-17. This unit is powered by a small, centrally-located electric motor which is geared to an outer traction wheel. Direction of motion is perpendicular to the axis of the unit. The traction wheel consists of two sections separated by a series of spacer bars which allow the aerosolizing air stream from the nozzle to pass freely through the traction wheel. A folding positioning arm drags on the ground behind the unit keeping the nozzle oriented downward at all times. The flexible aerosol transport tubes are attached to one end of the unit.

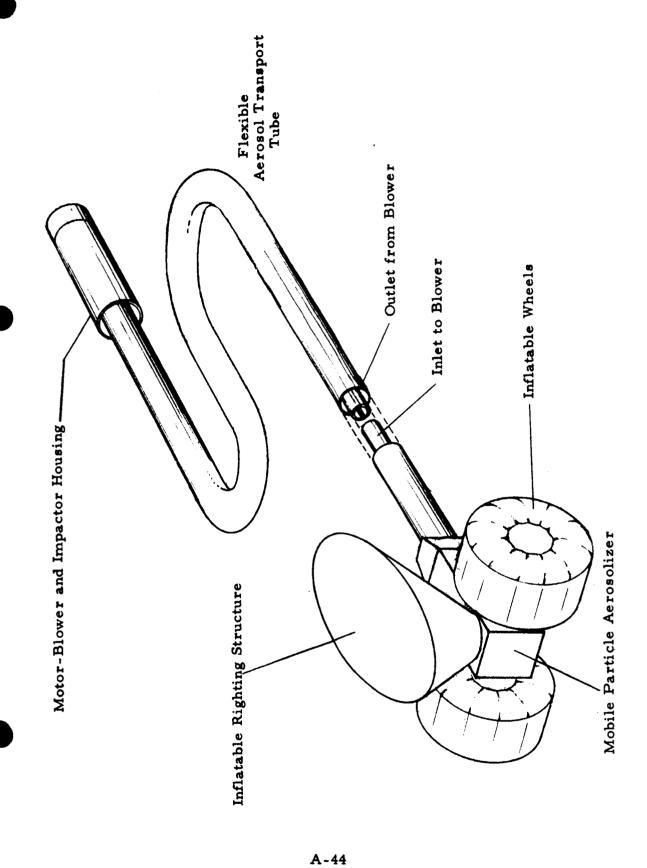


Figure A-16. Sample Collection Unit

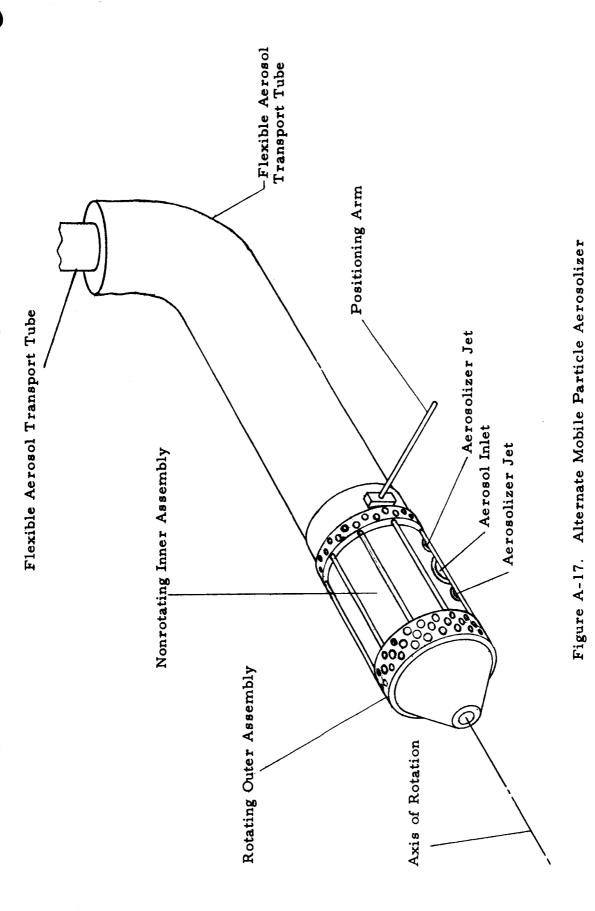


Figure A-18 is a stylized drawing of a unit that was designed in an effort to capitalize on the proven performance of the many track-laying vehicles that are employed in uncertain terrain. In order for the unit to fit inside the collector housing and at the same time have a sufficiently wide track base to assure good stability on the ground, the wheels were made to slide on their shafts. The wheels are pushed out to their fully extended position by four helical coil springs.

The flexible aerosol transport tubes are mounted on a member which hinges behind the unit when it is inside the cylindrical housing (Figure A-19). When the unit is ejected from the cylindrical housing, a spring pushes the motor up, and another spring brings the flexible aerosol transport tubes to a position between the tracks (Figure A-20).

An inflatable righting structure was fabricated for this unit. It was attached to the electric motor of the unit and served to right or turn over the aerosolizer unit when it landed upside down.

Preliminary tests showed that the inflatable structure would orient the unit to its correct position. Later we determined that the axial-flow blower would not supply sufficient pressure in a Martian environment to assure that the structure would operate as intended.

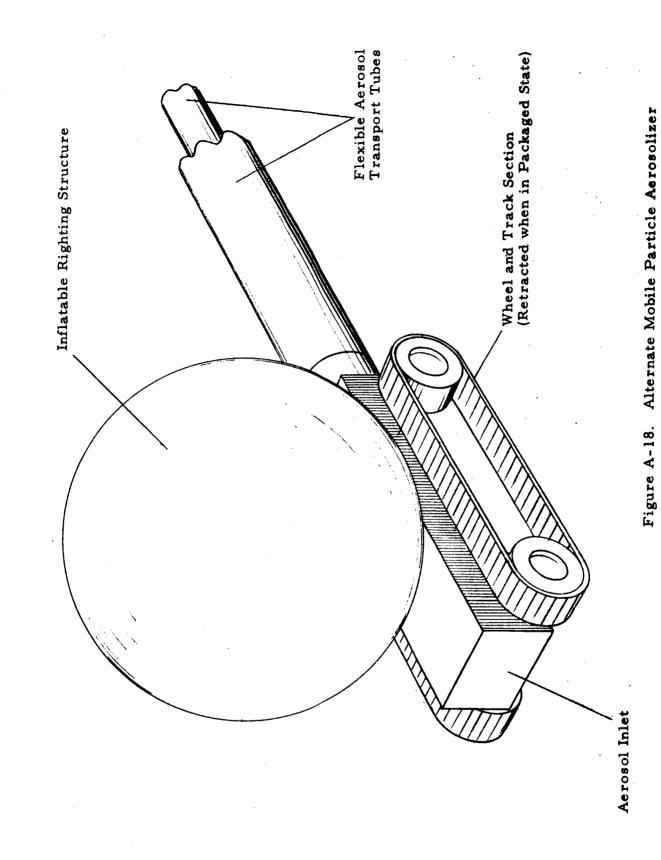
#### V. EVALUATION OF THE SAMPLE-COLLECTION UNIT

When completed, the breadboard sample collection unit and the two alternate mobile particle aerosolizer units were tested to evaluate 1) the mechanical performance of the mobile aerosolizer over various types of terrain,

2) efficiency of the impactor, 3) efficiency of impactor-transport tube assembly, and 4) the system's collection performance.

#### A. Mechanical Performance

As a result of the mechanical performance tests, the unit shown in Figure A-17 was eliminated from further consideration because it could not



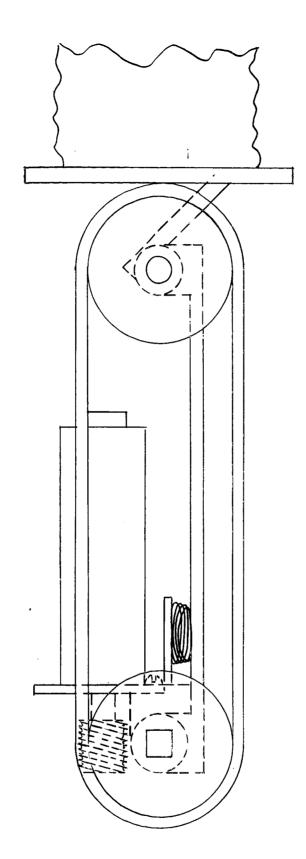


Figure A-19. Mobile Particle Aerosolizer in Folded Position

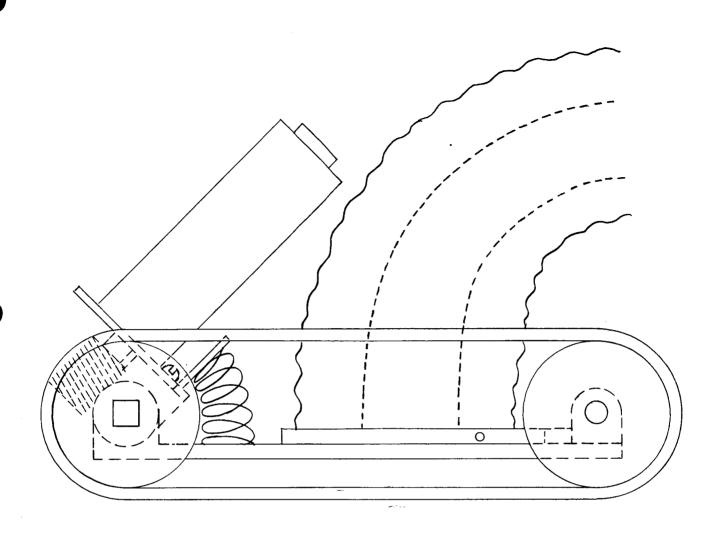


Figure A-20. Mobile Particle Aerosolizer in Extended Position

negotiate obstacles more than 1 inch high and because its mechanical operation was seriously impaired by sand and small rocks. The other two units performed approximately as expected and were deemed acceptable for further consideration although small design modifications were necessary.

## B. Impaction Efficiency

Impaction efficiency was determined by introducing known quantities of Arizona road dust or fine sand into the impactor nozzle and weighing the amount impacted. The collection efficiency for various runs ranged from 22 percent to 44 percent and averaged about 33 percent. The results for Arizona road dust (ARD) and fine sand were about the same. The efficiency data and several observations made at the time of the test are recorded below in Tables A-IX and A-X.

Table A-IX. Tests Using Arizona Road Dust

Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Quantity Sampled (g)	Percent Collected
0. 2391	0. 2662	0.0271	0.100	27
0. 2923	0. 3306	0.0383	0.100	38
0. 2702	0. 2855	0.0153	0.050	33

A very noticeable quantity of dust was evident in the blower effluent at the time just after the dust sample was admitted to the collector inlet. No quantitative evaluation of this dust was possible, but it appeared to be a substantial percentage of the total sample.

Inspection of the impactor's surface under the microscope indicated that the surface was not saturated with dust; hence it would have been able to entrain a larger percentage of the available dust had initial contact been made. The fact that only approximately 33 percent of either a 50 mg or a

100 mg sample was collected tends to substantiate the observation that a majority of the particles are not being impacted. Dust accumulated on the cavity walls between the nozzle and the impactor's surface indicates turbulence in this area.

A second series of tests were run using very fine sand rather than ARD.

Table A-X. Tests Using Fine Sand

Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Quantity Sampled (g)	Percent Collected
0. 2176	0.2395	0.0219	0.100	22
0. 2855	0.3149	0.0294	0.100	30
0. 2851	0.3271	0.0420	0.100	42

Run 4 was made using a collector surface prepared 3 days before the test, while runs 5 and 6 were made using surfaces prepared 30 minutes before the test. The latter two were much stickier than the first.

It is apparent from these tests that the impactor is capable of collecting approximately one-third of the very fine particles passing through the system. Tests conducted using course sand will indicate a very high collection efficiency provided the collection surface is sufficiently sticky to prevent particle re-entrainment.

## C. Impactor-Transport Tube Efficiency

The efficiency of the impactor-transport tube combination was determined in the same way as that of the impactor alone. The results of this test indicate a collection efficiency of about 15 percent for both fine sand and ARD.

## D. Sampler Performance Tests

A number of tests were conducted to determine the quantity of particulate material that could be collected from various surfaces in a given period of time by an aerosolizer having a given nozzle configuration. The impactor was positioned horizontally approximately 4 ft above and 6 ft away from the aerosolizer. The aerosolizer was held stationary at a predetermined distance above the terrain by a ring stand. The samples were collected on silicone grease coated gauze pads. Figure A-21 shows the results of two tests conducted with the aerosolizer outlet 1.5 and 3 inches above a surface of fine sand. The generally erratic nature of the data points for the 1.5-inch curve results from the fact that the pads were saturated within seconds after the test began. Figure A-22 shows the rate of collection for the two test conditions. It is clear from these curves that a given impaction surface will become saturated very rapidly and that large quantities of material could be collected by continually providing a clean impaction surface. For example, the 2-cm diameter impaction surface held about 90 mg of material, or approximately 30 mg per cm<sup>2</sup>. This quantity was collected in less than 30 seconds with the nozzle 1.5 inch above ground. At this rate, 1 gram of material could be collected in 6 minutes if the collection surface was replaced at a rate of 6 cm<sup>2</sup> per min.

During the evaluation of this sampling unit, several areas of less than optimum performance were observed. The cumulative effect of these is the 15 percent collection efficiency. Since the unit is capable of collecting such large quantities of sand in reasonable time periods, even at this low efficiency, no attempt to make a substantial improvement in efficiency was warranted.

#### VI. DESIGN OF THE PROTOTYPE SAMPLER UNIT

With the completion of the test program on the breadboard sampler, the design phase for the prototype sampler was initiated. Several design changes were suggested by the test program. These are discussed in the following paragraphs.

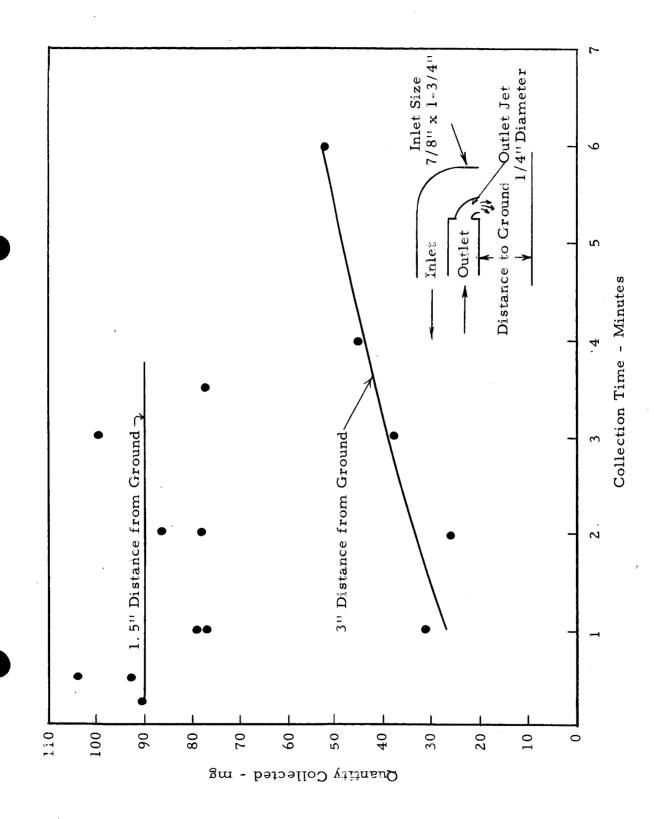


Figure A-21. Performance of Particle Collection System

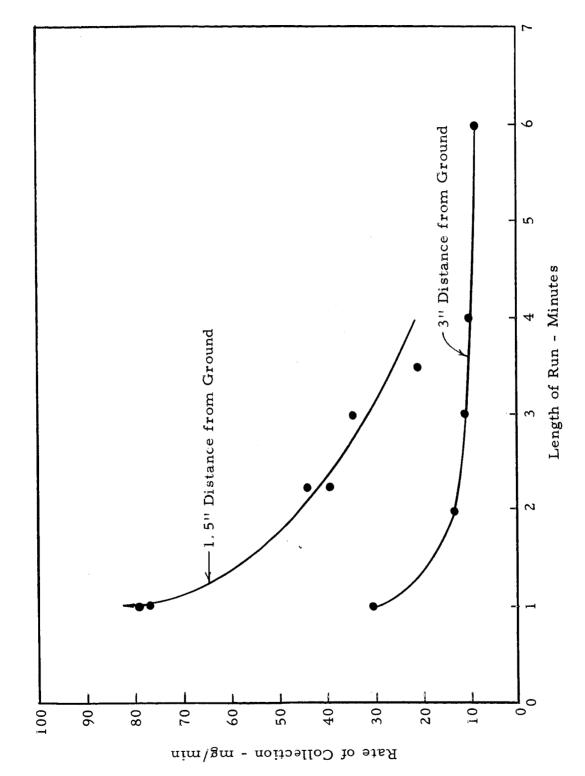


Figure A-22. Relation between Time and Rate of Collection

#### A. Aerosolizer

Figure A-23 shows an exploded view of the pneumatic-wheeled aero-solizer-collector unit. The primary difference between this model and the breadboard model is the dual collection feature, which enables the unit to collect from either side, depending on which side is down. Gravity serves to close the ports on the top side and open those on the bottom side. Because of this two-sided collection system, no righting mechanism is needed. Collection characteristics are the same as those of the earlier model.

A second design change involves the rigid, folding axel assembly. In the breadboard unit the tires were attached only to one hub and bore against the housing and upper righting structure; this allowed them to achieve a sufficiently large bending moment to keep properly oriented. Since the new model has neither the upper righting balloon nor sufficient housing area to provide a bearing surface, a rigid axel attached to both hubs is necessary. The axels fold back against the aerosolizer housing for packaging, and extend out and lock in a perpendicular position when the tires are inflated. The tires on this unit are fabricated from polyurethane-coated dacron and have lateral treads of a similar material. This fabric is sufficiently rugged to withstand any abrading it will receive in negotiating uneven terrain.

# B. Transport Tube

The external transport tube, which is also made from polyurethane-coated dacron, provides an extremely tough, puncture-resistant shield for the aluminized Mylar inner tube. This is important because a hole in the inner tube would seriously impair the function of the collector, whereas a hole in the outer tube would reduce its effectiveness only slightly. Both tubes are cemented to an aluminum flange that can be easily attached to any aerosolizer unit.

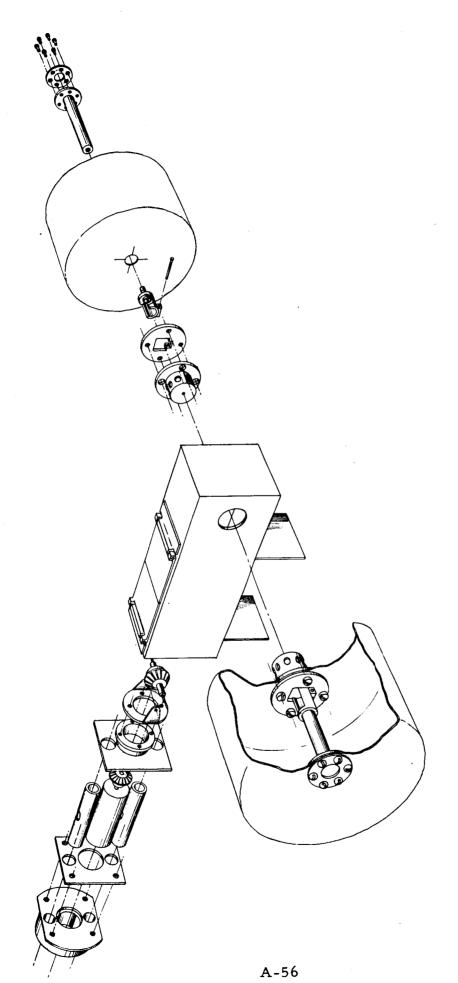


Figure A-23. Exploded View of Aerosolizer-Collector Unit (Prototype)

## C. Blower Section

A Globe Industries No. 53A105-1 motor was purchased and adapted for driving the Dean & Benson blower in a Martian atmosphere. This motor was smaller than the Hertner motor normally used in the Benson blower and required an adapter sleeve. The sleeve was made an integral part of the air-reversing section. This air reversing section causes air from the blower to flow outside of and in a direction opposite to the blower inlet air. An electrical connector was added at the blower end of the section to provide power leads to various sampler components.

The Globe motor will drive the Benson blower at Martian air density, but is not powerful enough to drive the blower at rated speed under Earth conditions. A second blower section, using a Hertner motor and Benson blower, was therefore designed and fabricated for test and evaluation purposes at Earth's air density. The second blower section is both mechanically and electrically interchangeable with the first.

## D. Impaction Section

The impaction section (Figure A-24) is easily removed from the sampler after the blower section has been removed from the nozzle section. The impactor section consists of a short tubular portion which divides the air flow (inlet air on inside and exit air on outside) and forms an air-tight connection between the nozzle and blower sections. There is a spring-clip arrangement to hold a gauze pad against the impactor plate. The actual specimen is collected on this pad.

#### E. Nozzle Section

The nozzle section (Figure A-24) includes the nozzle itself and the housing or main body of the sampler into which the transport tubes and aerosolizer are packed. The nozzle is 2 cm in diameter and has the central transport

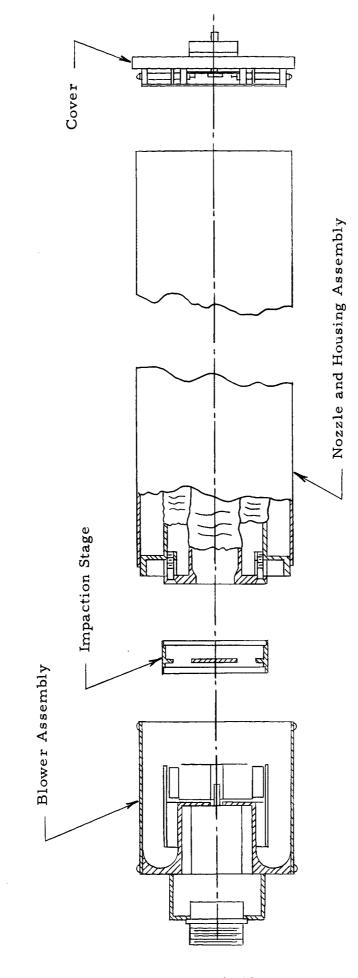


Figure A-24. Sectional View of Martian Sampling Unit

tube attached to it. The nozzle is mounted on spacers so that the blower's effluent air can flow around the outside of the nozzle and enter the space between the central and outer transport tubes.

## F. Cover Latching Mechanism

The cover was latched to the canister by means of two diametricallyopposite sliding pins which engaged holes in the canister. These pins were
spring loaded to be normally withdrawn from engagement. Their spacing
was such that when the armature of an electro-magnet was inserted between
the pins they would be held in engagement with the canister. Thus, when the
electro-magnet was energized and the armature was withdrawn, the pins
disengaged, and the cover flipped off with the aid of an auxiliary flat spring.

This mechanism, shown in Figure A-25, was used on the early models; but the final model employed a motorized cover and extraction device that are described later.

# G. Ejection Spring

We noted that when the transport tube and aerosolizer were packaged in the canister, a sizeable force was required to extract them. We therefore decided to investigate the use of a spring to drive the aerosolizer and transport tube out of the canister upon release of the cover. Use of a spring appeared to have several advantages, such as simplicity, light weight, no power required, no initiating or control functions required (other than release of cover), low cost, and reliability.

Two schemes were consequently devised, and one was built for trial and evaluation. The two are a coil-compression spring, and a constant-force spring.

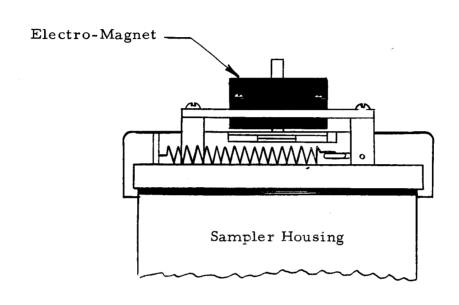


Figure A-25. Sampler Unit Cover and Latching Mechanism

# 1. Coil Spring

A coil spring was designed with a diameter that allowed it to slip easily over the outside of the transport tube and just slide freely inside the canister. A loose sleeve was provided to slide inside the spring to prevent folds of transport tube material from being pinched between coils of the spring. The mean diameter of the spring was 2.65 inches, and during packaging it was compressed from a free length of 10-1/2 inches to one of 3-5/8 inches.

A series of springs was made, to provide forces in the range of 1 to 9 lb at the compressed length.

We discovered during testing that even the strongest spring did not have enough force to overcome completely the friction between the coated fabric of the tires and the canister's walls. Several attempts were made (such as lubricants, slip liners, wrap-around restrainers, etc.) to reduce this friction, but none of them was completely successful.

# 2. Constant-Force Spring

A constant force spring or "Negator" (Hunter Spring Company) resembles in appearance the power spring used in clocks, but differs in design and application in that one end of the coil is attached to the linearly-moving load and the spring is forced to unwind from its normal tightly-wound condition about an axle.

Several samples of "Negator" springs were obtained from the Hunter Spring Company in the force range of approximately 1/2 to 40-1b tension.

Figure A-26 shows how we planned to accomplish extraction of the aero-solizer by using "Negator" constant-force springs. However, it was decided after evaluating the springs that this process could become somewhat complicated, and that there was a danger of the spring fouling while winding onto the axle.

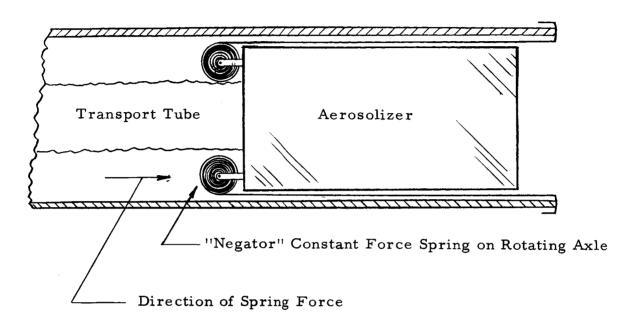


Figure A-26. Illustration of Application of "Negator" Constant Force Springs for Aerosolizer Extraction

We also found that springs having sufficient force to extract the unit were fairly bulky and increased the friction between the aerosolizer and housing wall.

## VII. SAMPLER EVALUATION AT LOW AIR DENSITY

A number of tests were conducted in an altitude chamber to evaluate the performance of the sampling unit at an air density approximately equivalent to that expected on Mars. The procedure used was similar to that used in the tests conducted at standard conditions (see page A-52). This testing was performed to allow a comparison of the sampler's performance characteristics under the two conditions. The aerosolizer-collector and the impactor were mounted so that they could be adjusted to any desired distance above the surface to be sampled. The pan containing the target material was mounted on a vibrating table to keep its surface level, thereby assuring that the distance between the aerosolizer and the surface would remain constant for the duration of the test. The samples were collected on gauze pads saturated with silicone grease. These were weighed before and after each test to determine the quantity collected. A number of runs were made with the aerosolizer positioned from 0 to 3 inches above the surface to determine the optimum position for the aerosolizer, and to determine the effect of this spacing on the quantity of sample collected. One run was also made at standard conditions to assure that the collector was still performing as reported earlier.

The data collected during this test are recorded in Table A-XI and are represented by the solid lines in Figure A-27. Some of the data reported in Figure A-21 are indicated by the dash line for purposes of comparison. It is evident from the low air-density curves that (all other factors equal) the sampler will collect essentially the same quantity of sand whether the blower is driven by the 5-watt Globe motor or the 12-watt Hertner motor. This was predicted from theory and from the motor performance tests. It is also evident that placing the aerosolizer less than 1 inch from the sand

Table A-XI. Data from Sampler-Performance Test

Ambient Type Pressure Sand (mb)	Ambient Pressure (mb)		Collector Height (inches)	Impactor Height (ft)	Tare Weight	Gross Weight (g)	Net Weight (g)	Time (min)
Globe	Lake	100	1	2.5	0.2959	0.3231	0.0272	4
Н-	Lake	100	_	2.5	0.3128	0.3500	0.0372	7
	Lake	100	1	2.5	0,3053	0.3494	0.0441	5.5
	Lake	100	1	2.5	0.3043	0.3643	0.0600	4
	Lake	100	0	2.5	0.2975	0,3105	0.0130	1
	Lake	100	1	2.5	0.3301	0.3816	0.0515	9
	Lake	100	Į.	2.5	0.3485	0.3642	0.0157	2
	Lake	100	1/8	2.5	0.3365	0.3436	0.0071	2.5
	Lake	1013	<b>с—</b>	2.5	0.3366	0.4140	0.0474	4
	ARD	100	1	2.5	, 0.3171	0.5095	0.1924	4
	ARD	100	1	2.5	0.3330	0.3928	0.0598	<b>=</b>
	ARD	100	<b>—</b>	2.5	0.3187	0.3494	0.0307	0.5
	ARD	100	П	2.5	0.3972	0.4147	0.0175	0.25
	Sand	100	<b>-</b>	2.5	0.3343			4
	Sand	100	П	2.5	0,4151	0.4490	0.0339	2
	Sand	100	1	2.5	0.3324	0.3629	0.0305	3

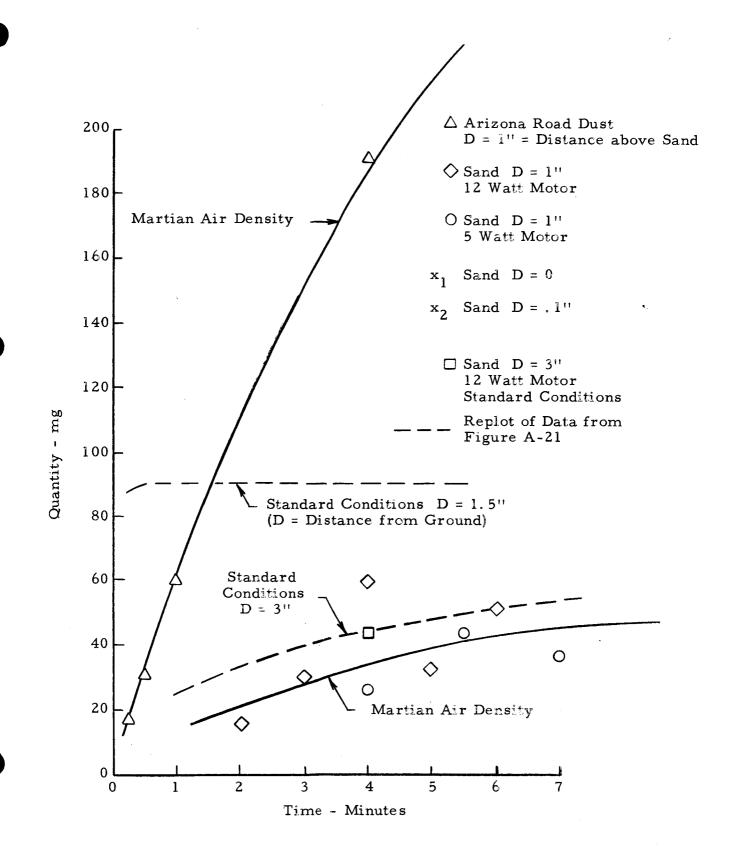


Figure A-27. Performance of the Particle Collection System

does not increase the quantity of sand collected per unit of time. This was not the case at standard conditions, where the sampler was saturated in less than 15 seconds at a distance of 1 to 1.5 inches. We observed after each run in the altitude chamber that more than 1 gram of sand had settled in the transport tube. This indicates that the aerosolizer-collector is functioning properly, but that there is not sufficient energy in the air-stream to keep the particles air-borne. Because the settling velocity of a particle is directly proportional to the gravitational force, it is reasonable to expect that several times as many particles will reach the impactor during sampling on Mars. This conclusion is further supported by the curve for Arizona road dust, where the particles are so small that essentially all of those picked up are transported to the impactor.

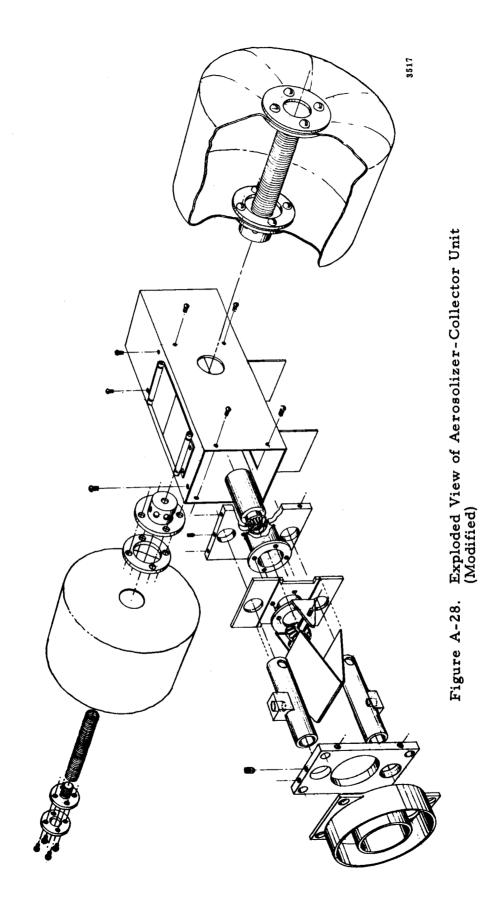
#### VIII. MODIFICATION OF THE PROTOTYPE SAMPLER UNIT

In addition to its collection performance, the mechanical performance of the sampler was tested extensively. As a result of these tests, some modification to improve its mechanical performance appeared desirable.

#### A. Aerosolizer Modification

Shifting the aerosolizer's center of gravity forward of the wheel was found to greatly improve the unit's ability to negotiate obstacles. This center of gravity shift was accomplished by replacing the drive motor with a shorter, lighter motor and by installing this motor ahead of the tires. The motor, gears, and associated mounting assemblies were housed in a reversed position to allow the axle to be moved near the center of the housing. Details of the modified aerosolizer are presented in Figure A-28.

A second change in the aerosolizer unit involved the folding axle. In the original design, the axles extended from a point near the forward end of the housing. Air pressure in the tires acting against the housing only at the after side of the tire thus produced a fore-aft bending moment in the axle. This



moment, combined with the vertical moment induced by the unit's weight, dictated that a rigid locking axle be used. When the axles were moved to a position near the center of the housing to improve balance, the fore-aft moment was eliminated. Therefore, an axle was required that was sufficiently rigid to overcome only the vertical bending moment. An axle assembly having no moving parts and incorporating a highly prestressed coil spring as the axle was built and tested. This assembly provided the required reliability and rigidity and allowed the axles to be folded against the housing for packaging.

## B. Aerosolizer Extractor Mechanism

It was originally intended that the aerosolizer be ejected from the canister by a spring arrangement. Several springs of two different types were tested. Test results indicated that, at best, the springs would barely force the aerosolizer clear of the canister. Because the springs were very unreliable and would not project the aerosolizer the desired distance from the canister, a more reliable ejection system was built. This system consists primarily of a printed-circuit d-c motor driving two ball-bearing jack screws. The ball nuts are mounted on opposite sides of the canister, whereas the motor and the ends of the two jack screws are mounted on the canister cover. The motor drives the screws by a roller chain and sprocket arrangement. Two fingers are mounted on the cover and extend into the canister, hooking behind the aerosolizer. When the cover is withdrawn from the canister by the motor and screws, the two fingers pull the aerosolizer from the canister and drop it to the ground. The assembly is shown in Figure A-29.

# C. Sample Collection Stage

The original sampler design provided collection of approximately 100 mg of material on a sticky surface mounted on the impaction plate. For testing and demonstrating the unit, it appeared desirable to provide both a larger collection capacity and easier access to the acquired sample. This was

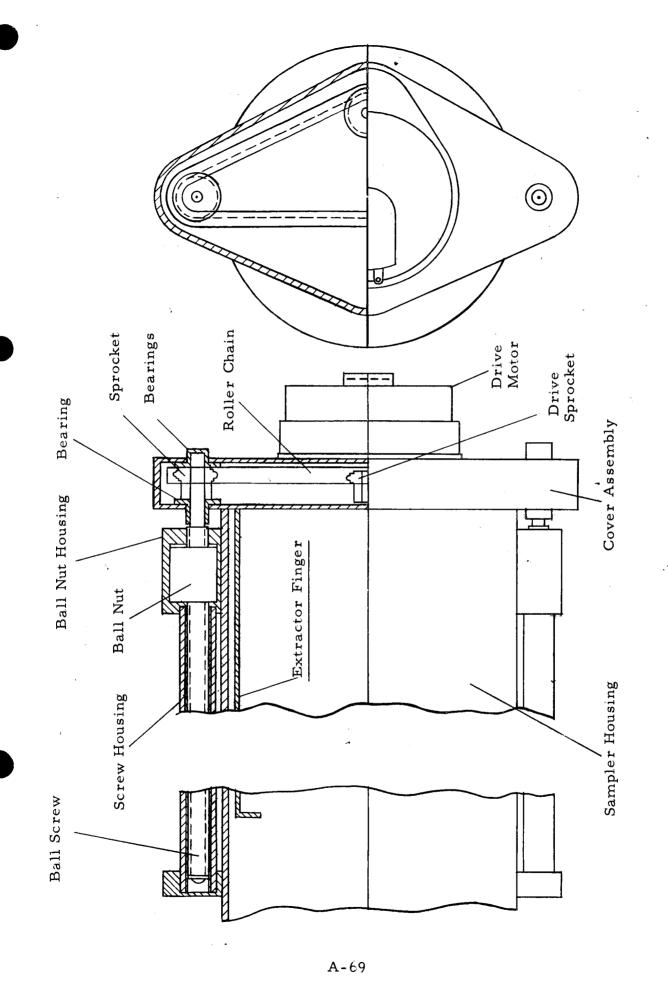


Figure A-29. Aerosolizer Extraction Assembly

accomplished by directing the air flow through an appendage where the particles are removed by a filter. Access to the collected sample is afforded by a door at the end of the external filter housing. Details of this device are shown in Figure A-30. This filter unit is completely interchangeable with the impactor stage, so no modification of the sample unit was required.

# D. Photographs of Modified Unit

Figures A-31 through A-35 are photographs of the sampler unit that embodies the above-mentioned modifications. The unit was tested and operated satisfactorily under laboratory conditions.

## IX. DRAWINGS

A complete set of drawings are included in Appendix C to comply with the contractual requirement for submitting shop drawings of the sampler unit.

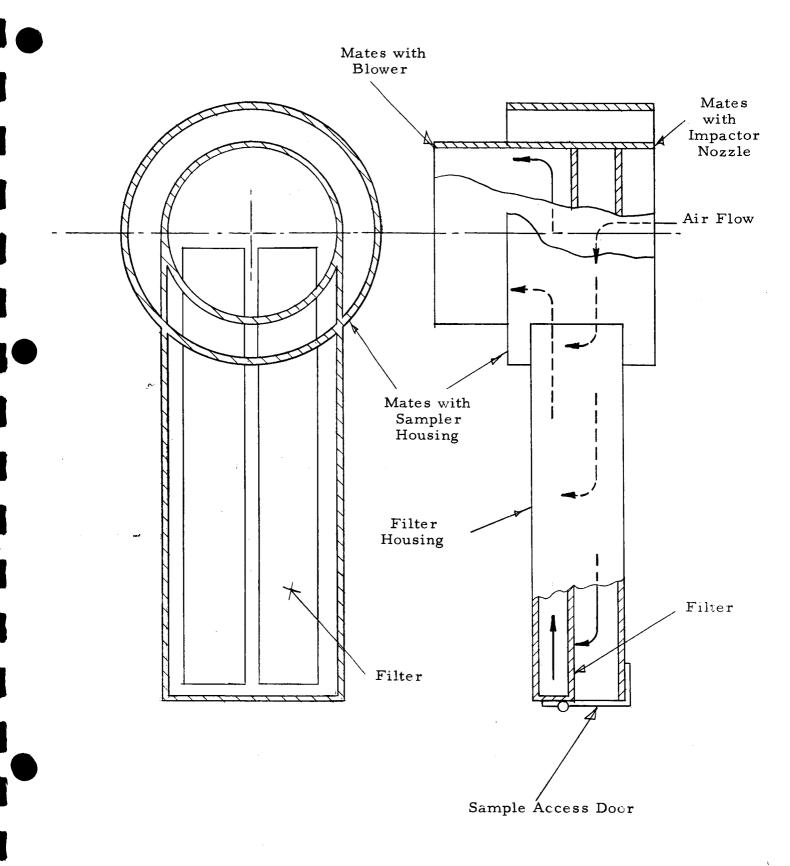


Figure A-30. Sample Collection Stage (Demonstration Model)

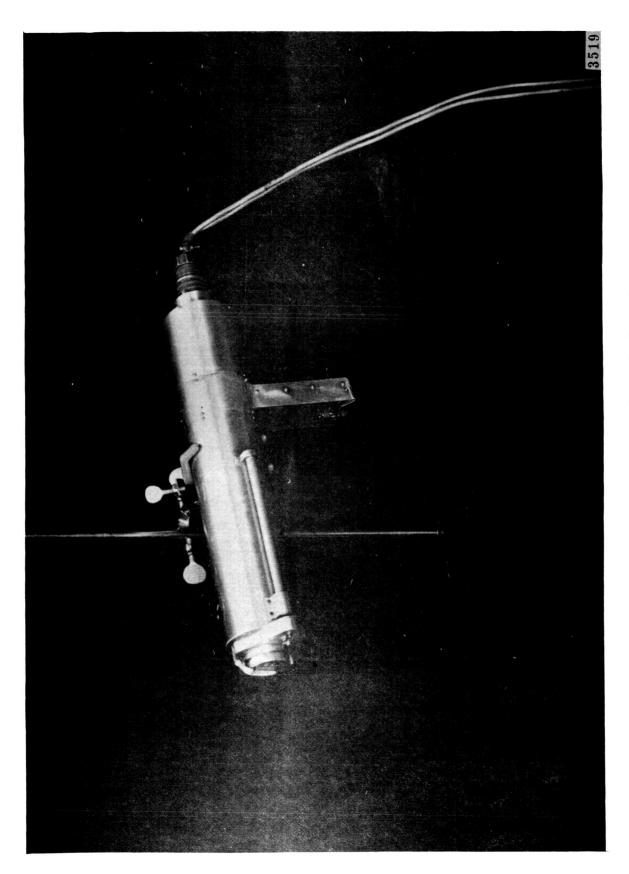


Figure A-31. Sampling Unit Packaged

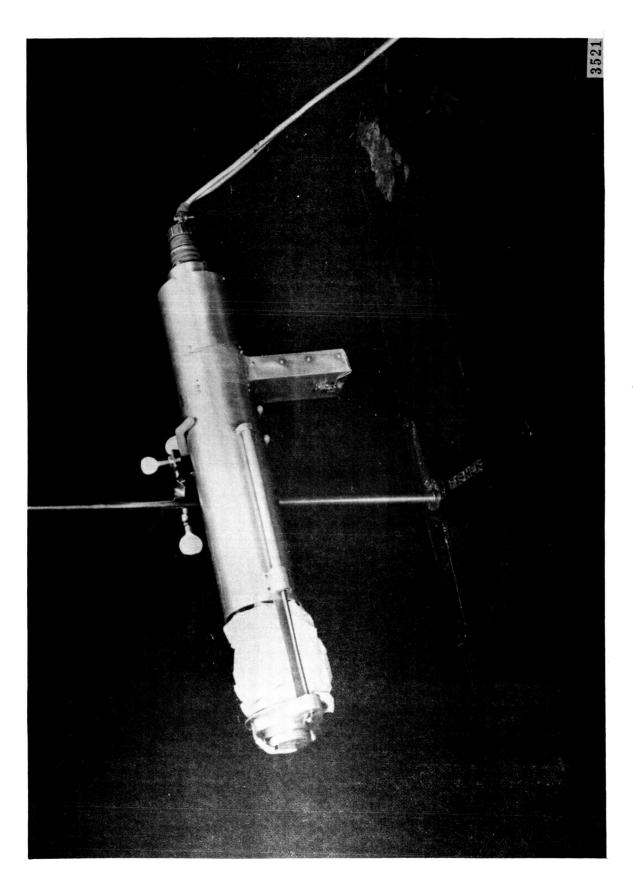


Figure A-32. Sampling Unit, Aerosolizer Being Extracted



Figure A-33. Sampling Unit, Aerosolizer Completely Extracted

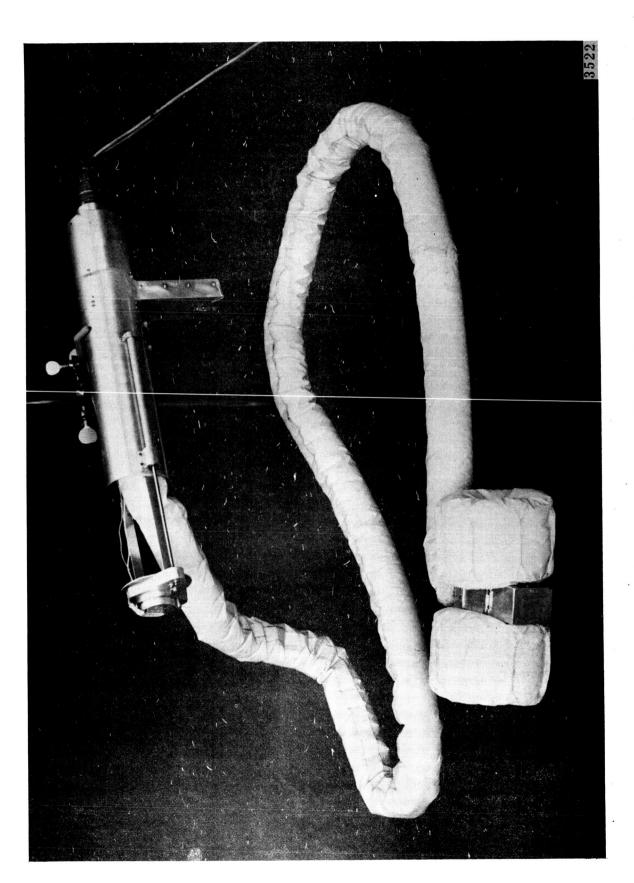


Figure A-34. Sampling Unit in Operation

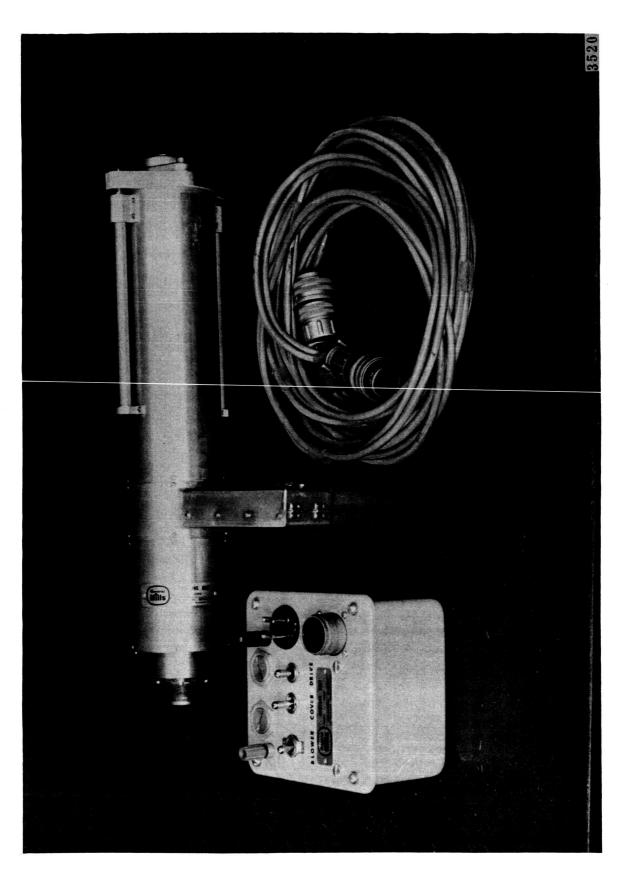


Figure A-35. Sampling Unit with Associated Control Box and Cable

APPENDIX B
BIOLOGICAL SUPPORT STUDY

## APPENDIX B

#### BIOLOGICAL SUPPORT STUDY

#### I. INTRODUCTION

During the eight months of this project's extension, our biological laboratory carried out investigations in the following areas:

- 1) Aerosolization and collection of soil microorganisms
- 2) Evaluation of various high density fluids that can be used for separating biological materials from dusts and soil by flotation
- 3) Determination of the minimal and optional quantities of flotation fluids for processing given quantities of dust and soil
- 4) Treatment of the dust and soil samples to facilitate purification of biological material by density flotation
- 5) Techniques for rapid detection of microorganisms in aerosolized dusts and soils.

In every phase of this work, the biological laboratory attempted only those experiments which would aid in the conception, design, fabrication, and operation of a sample collector-processor. Since this was not designed as a comprehensive and basic study of biological phenomena, many experiments which could have been conducted were not undertaken. Instead, the time limitations and the need of applying biological information to an engineering project directed our efforts to developing operational hardware that would be applicable to the problem at hand.

## II. AEROSOLIZATION STUDIES

Trials were performed to determine quantitatively the microorganism-particle size relationship in aerosols generated from different soil surfaces and the persistence of such aerosols. The laboratory arrangement is shown in Figure B-1. A given sample of soil was distributed over the surface of a table. The soil was aerosolized by the exhaust air (15 cfm) from a small blower mounted 12 inches above the table. Two different samplers were used in conjunction with this study. These were the Andersen sampler (1) and the Cassela sampler. (2) This work and all similar aerosolization studies were performed in our "clean room", where aerosols could be removed between trials by frequent air changes.

# A. Experiments to Determine Microorganism-Particle Size Relationship

The Andersen sampler was used in the experiments to determine quantitatively the microorganism-particle size relationship. The exhaust air from the blower was directed against the sample for a period of 3 minutes, and concurrently 3 ft<sup>3</sup> of air were sampled by the Andersen sampler (1 cfm). After incubation of the Andersen plates, we determined the relative number of organisms aerosolized and the particle-size distribution of the biological aerosol. These data are summarized in Table B-I.

The data suggest that a biologically rich aerosol can be generated from dry sands and soils with relatively low-velocity air streams, and that much of the biological matter is contained in the 4-micron and above size range of particles.

<sup>1.</sup> Andersen, A. A. New Sampler for the Collection, Sizing and Enumeration of Viable Air-Borne Bacteria, J. Bacteriol. 76: 471-484. 1958.

<sup>2.</sup> Bourdillon, R. B., O. M. Lidwell and J. C. Thomas. A Slit Sampler for Collecting and Counting Air-Borne Bacteria, J. Hygiene 41: 197-224. 1941.

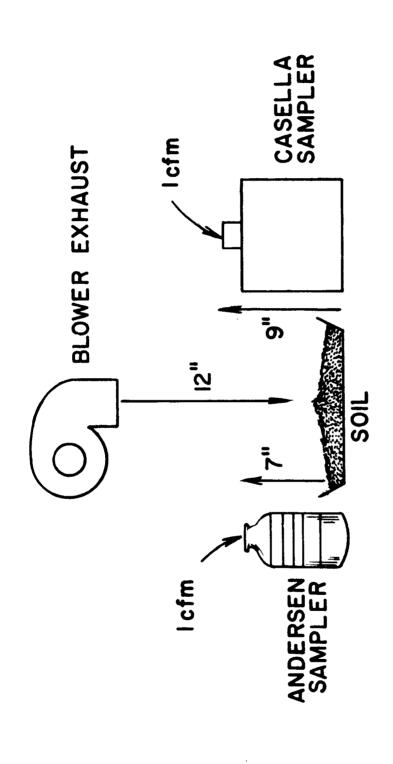


FIG. BI AEROSOLIZATION OF SOIL SAMPLE

Table B-I. Microorganism-Particle Size Relationship Obtained by Aerosolizing Various Dry Soils

Number of Microorganisms in a 3 ft<sup>3</sup> Air Sample

Type of	C						
Soil	>10, µ	6-10 µ	4-6 μ	2-4 μ	1-2 µ	< 1 μ	Total Count
Sand No. 1	47	24	15	11	5	0	102
Sand No. 2	420	160	97	24	14	20	735
Gravel	84	221	185	91	13	10	604
Asphalt	31	15	7	6	12	5	76
Mulch	53	72	61	93	326	516	1121
Field Soil	48	36	18	16	5	8	131

# B. Experiments to Determine Persistence of Aerosols

Further experiments were conducted to determine the persistence of aerosols generated from different soils. In these experiments, a Casella sampler sampling at a rate of 1 cfm for a 5-minute period was used together with an aerosolizer-blower which could be turned on during the sampling for varying periods of time ranging from 5 seconds to 5 minutes. The relative number of viable organisms per ft<sup>3</sup> which remain air borne was determined for each consecutive minute of the sampling period. These data are shown in Table B-II.

Table B-II. Persistence of Viable Organisms in Aerosols Generated from Various Dry Soils

Type of	Aerosolizer Blower -	Coun	t per ft <sup>3</sup> f	or Each Sa	ımpling M	inute
Soil	On Period	1st min	2nd min	3rd min	4th min	5th min
Sand No. 1	5 min	300	130	129	110	100
Sand No. 2	3 min	500	255	200	169	101
Loamy Soil	5 min	117	110	70	107	100
	3 min	140	110	67	75	47
Sandy Soil	3 min	20	16	6	18	12
	30 sec	11	13	21	28	24
	5 sec	7	12	8	8	14
Gravel No. 1	3 min	8	11	13	4	2
Gravel No. 2	3 min	8	8	6	9	8

These trials demonstrate that microorganisms aerosolized by blowing air against a dry surface persist in the air-borne state for a considerably period of time after the air blast terminates. They also demonstrate that the initial minute of sampling usually yields the highest count. This indicates the desirability of initiating the sampling process as soon as or even immediately before the aerosol is generated.

Several final experiments on aerosolization and collection are summarized in Table B-III. In all these trials, one-type of sand having a viable count of  $10^3$  organisms per gram was used. The variable tested was duration of the aerosolizing blast. Both particle-size distribution and persistence of airborne viable organisms were measured. (The experimental arrangement was again as shown in Figure B-1.)

Table B-III. Persistence and Particle-Size Distribution of Air-Borne Microorganisms from Aerosolized Sand

Aerosolizer				<b>-</b> .	_				r ft <sup>3</sup> ing M		
Blower -		Parti	cle Siz	e Inte	rval		lst	2nd	3rd	4th	5th
On Period	>10+	6-10	μ4-6μ	2-4µ	1-2µ	< 1 µ	min	min	min	min	min
5 sec	306	20	32	18	8	14	220	79	29	18	4
30 sec	555	101	138	44	3	4	145	81	46	21	12
l min	262	166	72	72	17	5	250	88	29	15	10
3 min	392	102	65	28	16	4	139	109	100	56	25
5 min		56	120	61	17	9	200	129	122	82	70
30 sec on, 30 sec off, for 3 min	108	169	37	39	11	27					
1 min on, 1 min off, for 3 min	342	102	61	22	38	10	200	127	96	90	12
Blower off	45		30	29	12	9	14	8	7	5	7

# III. EVALUATION OF FLOTATION FLUIDS

On the basis of theoretical reasoning, an ideal density-flotation fluid would have the following characteristics:

- 1) Density intermediate between the heavier microorganisms (≈1.5) and the lighter nonviable particles (≈2.0)
- 2) Low viscosity to permit rapid sedimentation of the dense fraction
- 3) Low surface tension to avoid indiscriminate trapping of particles at the air-liquid interface
- 4) Ability to retain biological material in suspension
- 5) Compatibility with the viability and/or enzymatic activity of microorganisms normally found in the environment
- 6) Non-interference with microscopic observation by visible light, phase contact, darkfield illumination, or ultraviolet.

Ideally, density flotation should simultaneously accomplish both purification (i.e., separation of biological from nonbiological particles) and concentration (i.e., retaining all of the biological fraction from a relatively large dust sample in a relatively small portion of liquid). Even if both objectives cannot be met in a single-step procedure, a suitable flotation fluid should permit their accomplishment by a two-step process: purifying in one liquid and concentrating the purified biological fraction in a smaller volume of another immiscible liquid, for which the biological fraction has a higher partition coefficient.

The criteria listed above describe an ideal fluid for a terrestial laboratory. In order to be useful for the Mars Microscope System, several other criteria must be met, among them stability, low volatility, low freezing point, ability to be sterilized, safety, etc.

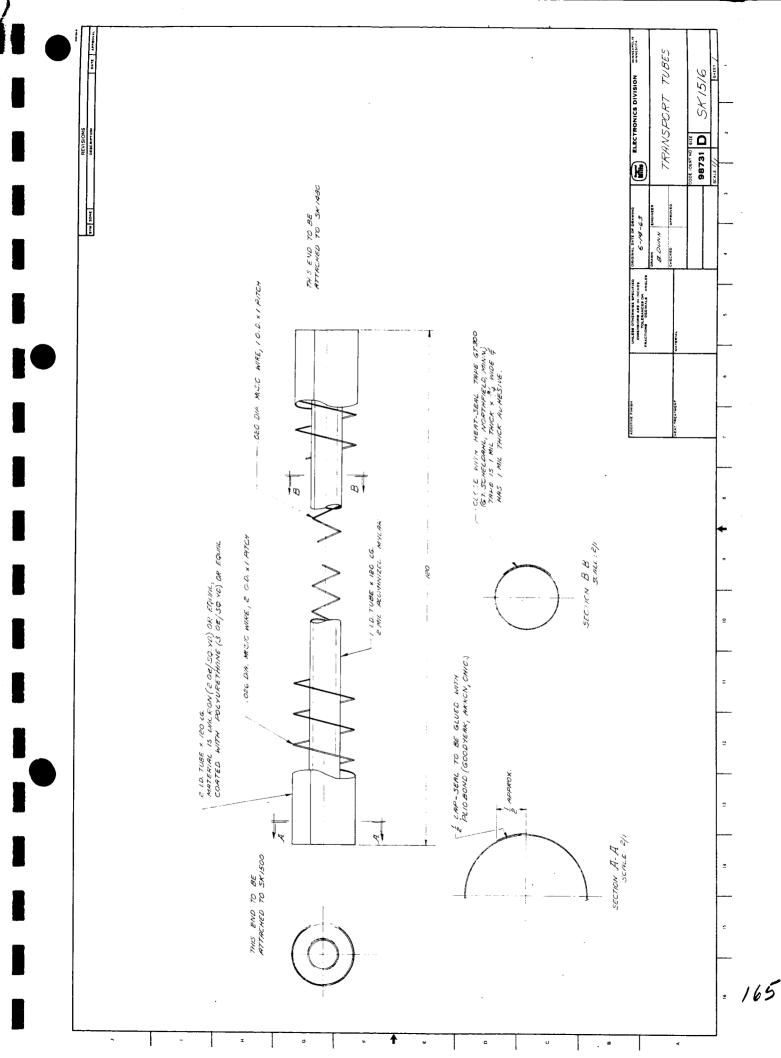
Table B-IV lists the flotation fluids evaluated during this study. Using these fluids, attempts were made to purify and concentrate the native microflora from a variety of dusts and soils, and to detect specific indicator organisms artificially inoculated in known concentrations. In certain trials the soils were added directly to the flotation fluid; in other trails the soils were aerosolized, then collected and purified. Most of the experiments involved culturing of microorganisms before and after purification, but some trials were based on microscopic counting of known biological particles artificially added.

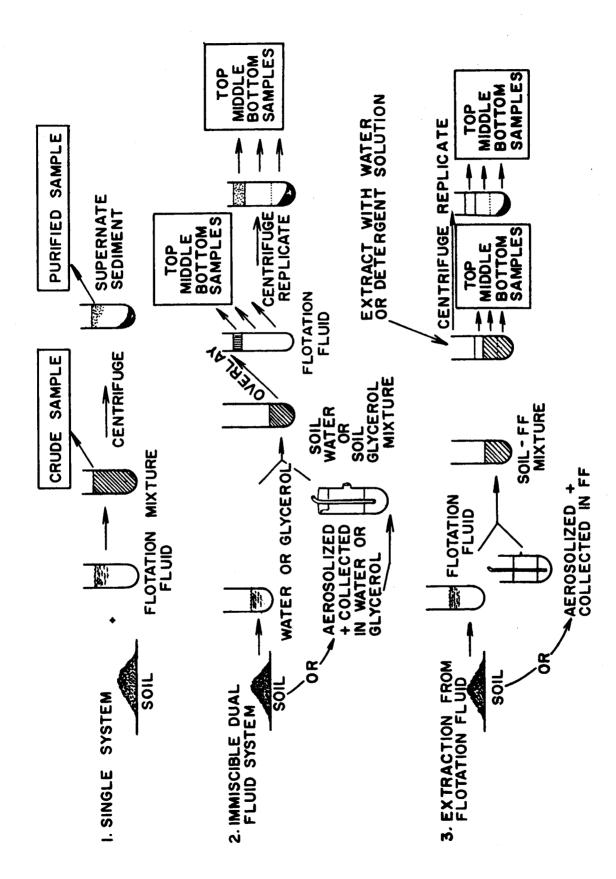
Since the fluids were evaluated by a variety of techniques during the course of several months, it would be inaccurate to compare one to another on the basis of the data presented. On the other hand, the data do permit an adequate evaluation of any given fluid under the condition of a given test procedure. Consequently, the laboratory techniques employed for three series of tests are presented in some detail below and diagramed in Figure B-2.

1) Single-System Flotation Experiments. The soil was mixed with the flotation fluid, and a sample was taken for culturing or microscopy. The system was then centrifuged until the supernatant was visibly clear. A sample of the supernatant was then cultured or microscopically examined. Representative data from these experiments are shown in Table B-V.

Table B-IV. Flotation Fluids Tested

Density	Density (g/ml)
Water	1.00
Glycerol	1. 26
Ludox HS (Colloidal Silica)	1.21
Glycerol-Ludox Mixtures	1.23
Carbon Tetrachloride	1.59
Carbon Disulfide	1.26
2-Bromopropene	1.31
Tricholoroethylene	1.46
Chloroform	1.49
Lead Nitrate (20 percent)	1. 20
Silver Nitrate (25 percent)	1. 25
Cadmium Iodide (25 percent)	1. 25
Cadmium Chloride (25 percent)	1. 26
Lactic Acid	1. 25
Pyruvic Aldehyde	1. 26
Lactate-Pyruvate Mixtures	1.25
Thioglycollic Acid	1.32
Silicone Oils	1. 20
Silicone Tetrachloride	1.52
Antimony Trichloride	1.52
Sodium Chloride (26 percent)	1.19
Magnesium Sulfate (20 percent)	1. 22
FC <sub>43</sub> (Fluorochemical)	1.77
FC <sub>75</sub> (Fluorochemical)	1.88
Kel F <sub>1</sub> (Polymer Oil)	1.84
Kel F <sub>3</sub> (Polymer Oil)	1. 93
Kel F <sub>10</sub> (Polymer Oil)	1. 96
2-Bromoethanol	1.77





USED BASIC FLOTATION TECHNIQUES OF THREE DIAGRAM FIG. B2

Table B-V. Single-System Flotation Experiments

Light Transmission	Supernatant (%)	92	06 <b>&lt;</b>	06<	06≺	80	85	85	Discolored	06≺	06 <b>~</b>	06 1	06▲	06▲	
Light T	Orude Mixture (%)	7	<50	<50	<b>4</b> 50	<50	<b>~</b> 50	<50	<b>~</b> 50	<b>V V V V V V V V V V</b>	<b>~</b> 50	<b>~</b> 50	<b>~</b> 50	<b>~</b> 50	
Count per Milliliter	Supernatant	110	70	20				1.0 ×	2.8	$1.5 \times 10^2$	<b>V</b> 10	۸10		$1.5 \times 10^3$	
Count pe	Crude Mixture	320	$3.0 \times 10^3$	380	250	$7.5 \times 10^3$	$1.6 \times 10^4$	$1.7 \times 10^4$	$5.0 \times 10^3$	$1.5 \times 10^4$	$2.2 \times 10^3$	$3.0\times10^3$	$3.0\times10^{3}$	$5.0 \times 10^3$	
	Centrifugation	10,000 rpm x 15 min	10,000 rpm x 15 min	10,000 rpm x 15 min	10,000 rpm x 15 min	5,000 rpm x 20 min	5,000 rpm x 20 min	5,000 rpm x 20 min	5,000 rpm x 20 min	5,000 rpm x 20 min	5,000 rpm x 20 min	5,000 rpm x 20 min	5,000 rpm x 20 min	5,000 rpm x 20 min	
	Quantity (g)	1.0	0.50	0.50	0.50	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	
	Soil	Arizona road dust (ARD)and lyophilized Serratia marcescens (Sm)	ARD	ARD	ARD	Clay and Bacillus globigii (Bg)	Clay and Bg	Clay and <u>Bg</u>	Clay and Bg	Clay and Bg	Clay and Bg	Clay and Bg	Clay and Bg	Clay and Bg	
	Quantity (ml)	50	50	20	20	50	50	20	10	10	10	10	10	10	
	Fluid	Ludox-Glycerol 1 to 1 Mixture	Water (control)	${ m MgSO}_{f 4}~(20\%)$	NaCl (26%)	g Glycerol (100%)	Glycerol (90%)	Glycerol (80%)	2-Bromopropene	$\mathtt{PbNO}_3$	$AgNO_3$	$CdI_2$	CdC1 <sub>2</sub>	CC14	gggggggg

Table B-V. Single-System Flotation Experiments (continued)

Light Transmission	Supernatant (%)	06 <b>A</b>	06 1	06	06	91
Light T	Crude Mixture (%)	<b>4</b> 50	<b>v</b>	<b>V</b>	<b>V</b> 50	<b>,</b>
Count per Milliliter	Supernatant	$1.5\times10^3$	$2.8 \times 10^2$	$2.8 \times 10^3$	$2.1 \times 10^3$	5.0 × 10 <sup>6</sup>
Count per	Crude Mixture	$5.0 \times 10^3$	$7.0 \times 10^3$	$5.0 \times 10^3$	$5.0 \times 10^3$	7.1 × 10 <sup>6</sup>
	Centrifugation	5,000 rpm x 20 min	5,000 rpm x 20 min	5,000 rpm x 20 min	5,000 rpm x 20 min	15,000 rpm x 15 min
	Quantity (g)	0.10	0.10	0.10	0.10	0.10
	Soil	Clay and Bg	Clay and Bg	Clay and Bg	Clay and Bg	ARD and rust spores (aero- solized)
	Quantity (m1)	10	10	10	10	R
	Fluid	CC14	$^2$ SO	Trichloro- ethylene	Chloroform	FC <sub>75</sub>

- Immiscible Dual-Fluid System. The soil was aerosolized and collected in either water or glycerol in a liquid impinger. The mixture was then layered over a dense, immiscible fluid, and aliquotes were taken from the top, middle, and bottom of the system for microbiological counts. Replicate tubes were then centrifuged. After the top layer had been visibly clarified, aliquotes of the system were again assayed for biological particles. Representative data from this series of experiments are shown in Table B-VI.
- 3) Extraction from Flotation Fluids. The soil was mixed with the flotation fluid (either directly or after aerosolization). The flotation fluid was then extracted with water or a detergent solution by shaking and permitting the aqueous layer to rise. Without further treatment, samples of the top (aqueous layer), middle (interface), and bottom (sediment) fractions of the tube were examined for biological particles. Replicate tubes were sampled after centrifuging. Representative data from this series of tests are shown in Table B-VII.

These data suggest that there are several feasible ways in which flotation may be incorporated into a purification-concentrating system for biological particles. For large entities such as rust spores, simple mixing of the aerosol with (or collecting the aerosol in) any fluid with the proper density ( $\approx 1.5$ ) followed by a brief centrifuging period was sufficient to concentrate a purified biological fraction at the meniscus. For the smaller microorganisms, which require significant purification and concentration before they can be observed, a dual-fluid system might be necessary. Extraction after a centrifugation process worked extremely well in the laboratory.

Investigation of the new high-density fluids (fluorochemicals and the polymer oils) has shown that they can be applied well to the problem of biological-particle purification. It appears from the literature on these materials that their physical and chemical properties would also render them promising for space-exploration work.

The most serious difficulty encountered with these materials is their inability to hold biological particles in true suspension. Otherwise they comply with all of the criteria outlined for ideal fluids.

Table B-VI. Immiscible Dual-Fluid Experiments

ssion lay on	After Cent.	(%)	62	83	87	95	98	95	82	95
Light Transmission in Overlay Fraction	Before Cent.		47	37	47	52	46	51	42	85
		Bottom			$3.0 \times 10^{5}$	$2.4 \times 10^{5}$	7		$2.7 \times 10^{3}$	$3.7 \times 10^3$
	After Centrifuging	Middle		`	1.4 × 10 <sup>6</sup>	1.2 × 10 <sup>6</sup>	30	$1.6 \times 10^3$	$9.1 \times 10^{4}$	$7.7 \times 10^4$
Count per Milliliter	Aft	Top	4.4 × 10 <sup>6</sup>	9.0 × 10 <sup>5</sup>	$1.5 \times 10^6$	$7.4 \times 10^{5}$	76	$4.3 \times 10^{3}$	$3.3 \times 10^{2}$	$3.0 \times 10^{5}$
Count per	uging	Bottom		ι	$1.4 \times 10^{5}$	1.6 × 10 <sup>5</sup>	٣		$2.8 \times 10^{3}$	$6.3 \times 10^4  3.4 \times 10^3$
	Before Centrifuging	Middle		1	6.8 x 10 <sup>5</sup>	3.0 × 10 <sup>5</sup>	30		$7.0 \times 10^4$	$6.3 \times 10^{4}$
	Bef	Top	4.7 × 106	1.6 × 10 <sup>6</sup>	3.8 × 10 <sup>6</sup>	1.7 × 10 <sup>6</sup>	80	$6.4 \times 10^3$	$4.6 \times 10^{5}$	3.5 x 10 <sup>5</sup>
		Centrifugation	15,000 rpm x 15 min	15,000 rpm x 15 min	15,000 rpm x 15 min	15,000 rpm x 15 min	15,000 rpm x 15 min	15,000 rpm x 15 min	15,000 rpm x 15 min	15,000 rpm x 15 min
		Soil	ARD and rust spores (aero-solized)	ARD and rust spores (aero- solized	ARD and sand and rust spores (aerosolized)	ARD and sand and rust spores (aerosolized)	ARD and Sm (aerosolized)	ARD and Sm (aerosolized)	ARD, sand, Bg (aerosolized)	ARD, sand, Bg (aerosolized)
	on on		2	7	2	7	7	7	7	7
	Quantity of Flotation	Fluid (ml)	FC <sub>75</sub>	$FC_{75}$	$FC_{75}$	$FC_{75}$	FC <sub>75</sub>	FC <sub>75</sub>	$FC_{75}$	FC <sub>75</sub>
	Ť.		-	-	-	-		7	1	1
	Quantity of Overlay	Fluid (ml)	Glycerol	н <sup>2</sup> о	Glycerol	н <sup>2</sup> о	Glycerol	н <sup>2</sup> 0	Glycerol	н20

Table B-VII, Extraction from Flotation-Fluid Experiments

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Quantity   Soil   Quantity   Channity   Ch					100		Befor	Before Centrifugation	ion	Afte	After Centrifugation	tion
10   ARD and Sm   0.2   0.1% Triton   5,000 rpm   8.3 x 10 <sup>4</sup>   3.2 x 10 <sup>5</sup>     10   Sand and Sm   0.2   0.1% Triton   5,000 rpm   1.7 x 10 <sup>6</sup>   3 x 10 <sup>3</sup>   1.9 x 10 <sup>5</sup>     3   ARD and Sm   0.1   0.1% Triton   12,000 rpm   1.8 x 10 <sup>5</sup>   5 x 10 <sup>3</sup>   1.9 x 10 <sup>3</sup>     4   Sand and Sm   0.1   0.1% Triton   12,000 rpm   1.8 x 10 <sup>5</sup>   5.2 x 10 <sup>2</sup>   5.4 x 10 <sup>4</sup>     5   Sand and Sm   0.1   0.1% Triton   12,000 rpm   1.8 x 10 <sup>5</sup>   5.2 x 10 <sup>2</sup>   5.4 x 10 <sup>4</sup>     5   Sand and Sm   0.1   0.1% Triton   12,000 rpm   12,000 rpm   12,000 rpm   12,000 rpm   12,000 rpm   13,000 rpm   12,000 rpm   13,000 rpm   10.8 x 10 <sup>6</sup>   1.8 x 10 <sup>6</sup>     6   Sand   0.1   1.0% Triton   12,000 rpm   1.8 x 10 <sup>6</sup>   5.2 x 10 <sup>6</sup>   8.5 x 10 <sup>6</sup>   5.4 x 10 <sup>6</sup>     7   Sand   0.1   1.0% Triton   12,000 rpm   1.8 x 10 <sup>6</sup>   5.8 x 10 <sup>6</sup>   5.2 x 10 <sup>6</sup>   8.5 x 10 <sup>6</sup>     8   Sand   0.1   1.0 m 1 H <sub>2</sub> O   15,000 rpm   1.8 x 10 <sup>4</sup>   1.6 x 10 <sup>6</sup>     9   ARD   1.0   1.0 m 1 H <sub>2</sub> O   15,000 rpm   1.8 x 10 <sup>4</sup>   1.6 x 10 <sup>6</sup>     9   ARD   1.0   1.0 m 1 H <sub>2</sub> O   15,000 rpm   1.8 x 10 <sup>4</sup>   1.6 x 10 <sup>6</sup>     9   ARD   1.0   1.0 m 1 H <sub>2</sub> O   15,000 rpm   1.8 x 10 <sup>4</sup>   1.6 x 10 <sup>6</sup>     9   ARD   1.0   1.0 m 1 H <sub>2</sub> O   15,000 rpm   2.2 x 10 <sup>4</sup>   1.7 x 10 <sup>3</sup>     10   1.7 x 10 m 1   1.0 m	Fluid	Quantity (ml)	Soil	Quantity (g)	Extraction	Centrifugation	Top		- i	Top	Middle	Bottom
10   Sand and Sm   0.2   0.1% Triton   5,000 rpm   1.7 × 10 <sup>6</sup>   5.0 × 10 <sup>5</sup>     3   ARD and Sm   0.1   0.1% Triton   12,000 rpm   1.7 × 10 <sup>4</sup>   3 × 10 <sup>3</sup>   1.9 × 10 <sup>5</sup>     3   Sand and Sm   0.1   0.1% Triton   12,000 rpm   1.8 × 10 <sup>5</sup>   5.10 <sup>3</sup>   5.4 × 10 <sup>4</sup>     4   Sand   1.0   1.0% Triton   12,000 rpm   1.8 × 10 <sup>5</sup>   5.2 × 10 <sup>2</sup>   5.5 × 10 <sup>3</sup>   3.0 × 10 <sup>3</sup>   1.8 × 10 <sup>3</sup>     4   Sand   0.1   1.0% Triton   12,000 rpm   4.4 × 10 <sup>4</sup>   4.5 × 10 <sup>5</sup>	FC <sub>75</sub>	10	ARD and Sm	0.2	0.1% Triton X-100 - 2 ml	æ	$8.3 \times 10^4$			3. 2 × 10 <sup>5</sup>		
3 Sand and Sm 0.1 0.1% Triton 12,000 rpm 7 x 10 <sup>4</sup> 3 x 10 <sup>2</sup> 1.9 x 10 <sup>2</sup> 3 Sand and Sm 0.1 2,100 rpm 1,18 x 10 <sup>5</sup> 5 x 10 <sup>2</sup> 5 x 10 <sup>4</sup> 5 x 10 <sup>4</sup> 4 Sand 0.1 1.0% Triton 12,000 rpm 5.3 x 10 <sup>2</sup> 8.2 x 10 <sup>2</sup> 6.5 x 10 <sup>2</sup> 3.0 x 10 <sup>3</sup> 1.8 x 10 <sup>3</sup> 4 Sand 0.1 1.0% Triton 12,000 rpm 44  5 Sand 0.1 1.0% Triton 12,000 rpm 44  Sand 0.1 1.0% Triton 12,000 rpm 108  7 X-100 - 1 ml x 10 min 108  3 ARD and rust solized 1.0 ml H <sub>2</sub> O 15,000 rpm 1.5 x 10 <sup>7</sup> 5.8 x 10 <sup>6</sup> 2.2 x 10 <sup>6</sup> 8.5 x 10 <sup>6</sup> 5.4 x 10 <sup>6</sup> 1 3 ARD 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 1.8 x 10 <sup>4</sup> 1 3 ARD 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 6.2 x 10 <sup>4</sup> 1 3 ARD 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 6.2 x 10 <sup>4</sup> 1 1	FC <sub>75</sub>	10	Sand and Sm	0.2	0.1% Triton X-100 - 2 ml		1.7 × 10 <sup>6</sup>			5.0 × 10 <sup>5</sup>		u
3 Sand and Sm	$FC_{75}$	8	ARD and Sm	0.1	0.1% Triton X-100 - 1 ml	12,000 rpm x 10 min	$7 \times 10^4$			$1.9 \times 10^{3}$		1.1 × 10 <sup>2</sup>
4 Sand 1.0 1.0% Triton 12,000 rpm 5.3 x 10 <sup>2</sup> 8.2 x 10 <sup>2</sup> 6.5 x 10 <sup>2</sup> 3.0 x 10 <sup>3</sup> 1.8 x 10 <sup>3</sup> 4 Sand 0.1 1.0% Triton 12,000 rpm 44  5 Sand 0.1 1.0% Triton 12,000 rpm 108  7 X-100 - 1 ml x 10 min    8 X-100 - 1 ml x 10 min    1.0 ml H <sub>2</sub> O 15,000 rpm 8 x 10 <sup>2</sup> 8 X 10 <sup>2</sup> 8.5 x 10 <sup>6</sup> 8.5 x 10 <sup>6</sup> 5.4 x 10 <sup>6</sup> 1.0 ml H <sub>2</sub> O 15,000 rpm 1 8 x 10 <sup>2</sup> 1.0 ml H <sub>2</sub> O 15,000 rpm 1 8 x 10 <sup>2</sup> 1.0 ml H <sub>2</sub> O 15,000 rpm 1 8 x 10 <sup>4</sup> 1.0 ml H <sub>2</sub> O 15,000 rpm 1 8 x 10 <sup>4</sup> 1.0 ml H <sub>2</sub> O 15,000 rpm 2.2 x 10 <sup>4</sup> 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 2.2 x 10 <sup>4</sup> 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 2.2 x 10 <sup>4</sup> 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 2.2 x 10 <sup>4</sup> 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 2.2 x 10 <sup>4</sup> 1.7 x 10 <sup>3</sup> 1.8 x 10 <sup>4</sup> 1.7 x 10 <sup>3</sup> 1.8 x 10 <sup>4</sup> 1.7 x 10 <sup>3</sup>	$FC_{75}$	ъ	Sand and Sm	0.1	0.1% Triton X-100 - 1 ml	12,000 rpm x 10 min				$5.4 \times 10^{2}$	r	4 × 10 <sup>2</sup>
4 Sand 0.1 1.0% Triton 12,000 rpm 44 97 1  2 ARD and rust solized 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 1.8 x 10 <sup>2</sup> 3 ARD 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 1.8 x 10 <sup>2</sup> 1 3 ARD 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 1.8 x 10 <sup>2</sup> 3 ARD 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 6.2 x 10 <sup>4</sup> 1 3 ARD 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 6.2 x 10 <sup>4</sup> 1 3 ARD 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 6.2 x 10 <sup>4</sup> 1 3 ARD 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 6.2 x 10 <sup>4</sup> 1 4 Sand 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 6.2 x 10 <sup>4</sup> 1 5 ARD 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 6.2 x 10 <sup>4</sup> 1 6 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 7.2 x 10 <sup>4</sup> 1 7 x 10 <sup>3</sup> 1 7 x 10 <sup>3</sup>	$FC_{75}$	4	Sand	1.0	1.0% Triton X-100 - 1 ml	12,000 rpm x 10 min				3.0 × 10 <sup>3</sup>	$1.8 \times 10^{3}$	5.0 × 10 <sup>4</sup>
4 Sand 0.1 1.0% Triton $1.0,000$ rpm $1.08$ $1.08$ $1.21$ 5.0 $5.0$ $1.$	$FC_{75}$	4	Sand	0.1	1.0% Triton X-100 - 1 ml	12,000 rpm x 10 min	44			26	1	117
3 ARD and rust 1.0 ml H <sub>2</sub> O 15,000 rpm 1.5 x 10 <sup>7</sup> 5.8 x 10 <sup>6</sup> 2.2 x 10 <sup>6</sup> 8.5 x 10 <sup>6</sup> 5.4 x 10 <sup>6</sup> solized 2.2 x 15 min 8 x 10 <sup>2</sup> 9 x 15 min 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 6.2 x 10 <sup>4</sup> 1.6 x 10 <sup>3</sup> 1.6 x 10 <sup>3</sup> 1.6 x 10 1.0 ml H <sub>2</sub> O 15,000 rpm 2.2 x 10 <sup>4</sup> 1.6 x 10 <sup>5</sup> 1.7 x 10 <sup>3</sup> 1.7 x 10	$FC_{43}$	4	Sand	0.1	1.0% Triton X-100 - 1 ml	12,000 rpm x 10 min				121	20	84
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	FC <sub>7-5</sub>	8	ARD and rust spores (aero- solized		1.0 ml H <sub>2</sub> O	15,000 rpm x 15 min				8.5 × 10 <sup>6</sup>		3.0 × 10 <sup>6</sup>
3 ARD 1.0 1.0 ml $H_2^{O}$ 15,000 rpm 1.8 x 10 <sup>4</sup> x 15 min 3 ARD 1.0 1.0 ml $H_2^{O}$ 15,000 rpm 6.2 x 10 <sup>4</sup> x 15 min 3 ARD 1.0 1.0 ml $H_2^{O}$ 15,000 rpm 2.2 x 10 <sup>4</sup> x 15 min	2-Bromo- ethanol		ARD	1.0	1.0 ml H <sub>2</sub> O	15,000 rpm x 15 min	$8 \times 10^2$			8 x 10 <sup>2</sup>		
3 ARD 1.0 1.0 ml $H_2$ O 15,000 rpm $6.2 \times 10^4$ $\times$ 15 min 3 ARD 1.0 1.0 ml $H_2$ O 15,000 rpm $2.2 \times 10^4$ $\times$ 15 min	Kel ${\tt F}_1$	3	ARD	1.0	$1.0 \text{ ml H}_2^{\text{O}}$	15,000 rpm x 15 min	$1.8 \times 10^4$			1.6 × 10 <sup>3</sup>		
3 ARD 1.0 1.0 ml H <sub>2</sub> O 15,000 rpm 2.2 x 10 <sup>4</sup> x 15 min	Kel F <sub>3</sub>	3	ARD	1.0	1.0 ml H <sub>2</sub> O	15,000 rpm x 15 min	$6.2 \times 10^{4}$			1.6 × 10 <sup>5</sup>		
	Kel F <sub>10</sub>	3	ARD	1.0	$1.0 \text{ ml H}_2\text{O}$	15,000 rpm x 15 min	$2.2 \times 10^4$			$1.7 \times 10^3$		

## IV. DETERMINATION OF MINIMUM VOLUMES OF FLUIDS REQUIRED FOR FLOTATION

Once the flotation process had been demonstrated as feasible in principle, it was necessary to obtain information about the appropriate volumes of flotation fluids and overlay fluids required for the process. A series of experiments was performed, testing various ratios of fluids and various quantities of soil; these data are summarized in Table B-VIII.

It should be pointed out that these values are based on "batch" centrifutation trials in individual sample tubes. Consequently, any losses of biological material on the walls of the tubes, which were not recovered in the extraction step, could result in a significant variation that would not necessarily be encountered in a continuous separation process. This loss would be particularly marked when extremely small quantities of extraction fluid were employed.

Nevertheless, these trials led to the following conclusions:

- 1) Satisfactory flotation can be accomplished when 3 ml of fluorochemical are used to process 1 g of soil;
- 2) This volume of fluorochemical after centrifugation can be easily extracted with as little as 0.01 ml H<sub>2</sub>O;
- 3) The only advantage gained by using more flotation or extraction fluid is convenience of handling in the laboratory.

## V. TREATMENT OF SAMPLE TO FACILITATE FLOTATION

Under natural conditions, the microflora of soils and dusts are intimately associated with and often tightly attached to inorganic particles. Therefore, any purification system which attempts to separate inorganic particles from biological particles on the basis of density alone will be handicapped by the sedimentation of much of the biological fraction which adheres to the mineral fragments. Many attempts were made in our laboratory to dislodge the microorganisms from the inanimate particles to which they are attached. The rationale was to generate a biological-nonbiological suspension which could then be fractionated by density flotation.

Recovery of Biological Particles from Soils as a Function of Flotation Fluid and Overlay Fluid Volumes Table B-VIII.

Purification Technique	Soil	Quantity (g)	Flotation Fluid	Quantity (m1)	Overlay Fluid (ml)	Count per Milliliter of Biological Material in Overlay Fluid
Soil and FC75 mixed and extracted with water; no centrifuging	Sand	0.1	FC <sub>75</sub>	1. 0 2. 0 4. 0	1.0 1.0	$2.8 \times 10^{3}$ $1.0 \times 10^{3}$ $1.0 \times 10^{3}$
		1.0	FC <sub>75</sub>	1. 0 2. 0 4. 0	1.0 1.0 1.0	8. $0 \times 10^2$ 1. $0 \times 10^3$ 1. $6 \times 10^3$
Soil and FC <sub>75</sub> mixed and extracted with water, total system centrifuged at	ARD and rust spores	0.1	$FC_{75}$	0.5	1.0	4. 2 x 106 4. 5 x 106 2. 6 x 106
15,000 rpm × 15 min		1.0	$FC_{75}$	1.0 3.0 5.0	1.0	1. $4 \times 10^7$ 9. $6 \times 10^6$ 1. $7 \times 10^7$
Soil aerosolized and collected in flotation fluid; extracted with water; total system centrifuged at 15,000 rpm x 15 min	ARD and rust spores (aero- solized)		FC75	6.6.6. 0.00	1. 0 0. 5 0. 1 0. 01	1. $1 \times 10^7$ 1. $1 \times 10^7$ 4. $6 \times 10^6$ 7. $2 \times 10^5$
Soil and FC75 mixed and centrifuged at 15,000 rpm x 15 min; extracted with water after centrifuging	ARD	0.10010010010010010010010010010010010010	FC <sub>75</sub>	5.0000	00000	1.5 x 106 1.0 x 105 5.0 x 104 5.6 x 104 3.8 x 104 4.5 x 104
Soil and FC <sub>75</sub> mixed and centrifuged at 15,000 rpm x 15 min; extracted with water after centrifuging	ARD	1. 0	FC <sub>75</sub>	9. 9. 9. 0. 9. 0.	1. 0 0. 5 0. 1 0. 01	4.3 x 106 3.8 x 105 3.3 x 105 3.2 x 105

Essentially two basic treatments were employed: Ultrasonic vibration, and the use of detergents. A summary of these trials is presented as Table B-IX. It was possible to increase the viable count in the treated samples by a combination of detergent and ultrasonic application. However, the increased yields are probably not worth the expenditure of energy in a power-limited Martian probe. Other trials showed that if these treatments are not carefully controlled, germicidal effects occur.

## VI. RAPID DETECTION STUDIES

Several miscellaneous experiments were performed in the area of biochemical and microscopic detection of bacteria after purification. It was possible to pass the aqueous overlay from flotation systems through membrane filters after centrifugation and to stain the microorganisms directly on the filter. Using known organisms of definite and recognizable morphology (e.g., Sarcinal and Streptococci), it was easy to demonstrate the relative degree of purification accomplished. It was extremely difficult, however, to detect microscopically the presence of indigenous organisms less than 5 microns in diameter from native soils, even after purification. Mold spores and rotifier microcysts (>5-micron diameter) from these soils were easily recognized.

In certain trials, resazurin or tetrazolium solutions were used as extraction fluids in an immiscible flotation system. With soils containing approximately 10<sup>4</sup> organisms per gram, color changes in the clear supernatant were evident after 2.5 hours. Although this work was not extensively pursued, we believe that there is promise in a biochemical detection system in which the microbial fraction of soils and dusts can be retained in a small volume of indicator medium purified to a light transmission of >90 percent.

Table B-IX. Effect of Various Treatments on Facilitating Recovery of Biological Material from Soils

			Viable Count per Milliliter	per Milliliter
Soil	Fluid System	Treatment	Before Centrifuging	After Centrifuging
Sand	Н,О	None	$9 \times 10^3$	
	<b>J</b>	Ultrasonic (40 kc $\times$ 15 min)	$4.7 \times 10^4$	
		0.1% T 20	$8.0 \times 10^{3}$	
		Ultrasonic + 0.1% T 20	$7.7 \times 10^4$	
		1.0% T 20	$2.6 \times 10^4$	
		Ultrasonic + 1.0% T 20	$1.5 \times 10^{5}$	
ARD	Ludox Glycerin	None	1180	009
		0.1% T 20	620	360
		1.0% T 20	550	150
		Ultrasonic (40 kc x 15 min)	1290	1000
		Ultrasonic + 0.1% T 20	069	270
		Ultrasonic + 1.0% T 20	870	200
Sand and Sm	Glycerol over FC <sub>7E</sub>	None	10	26
I	2	Ultrasonic (40 kc x 15 min)	42	150

## VII. SIZE FRACTIONATING BIOLOGICAL MATERIAL

A study was made of ways to fractionate the biological material into several different particle size ranges for the purpose of facilitating microscopic examination. The size ranges chosen were >100 microns, 100 to 10 microns, and <10 microns.

The biological material used in these experiments was a mixture of rust spores, mold spores, and <u>Bg</u> that had been purified from inorganic particulate contamination by means of the density-flotation procedure described previously. After purification, the biological material was removed from the top of the suspension liquid (FC<sub>75</sub>) by resuspending it in an overlay of water.

The approach taken was to filter a sample of the purified biological material suspended in water through two successive screens having nominal opening sizes of 100 and 10 microns. The actual opening sizes determined by microscopic measurement were 95 x 80 microns for the coarse screen and 10 x 10 microns for the fine one. The filtration apparatus is illustrated in Figure B-3.

Because of its high surface tension, the water did not pass readily through the fine screen. Several attempts were made to rectify this problem. These were 1) adding a detergent to the water, 2) centrifuging the screen holder at speeds up to 1800 rpm, 3) vibrating the screen holder at 60 cps, 4) connecting the lower end of the screen holder to a vacuum system (water aspirator), and 5) replacing the 10-micron screen with a 15-micron screen. Of these measures, only the latter two were satisfactory.

In the particular sample of biological material analyzed, there were no particles larger than 100 microns. All particles therefore passed through the first screen. Essentially all particles except the rust spores passed through the second screen, whether the screen's opening was 10 or 15 microns. The reason for this was that there were no particles in the 5 to 20-micron size range.

These experiments demonstrate the feasibility of size-fractionating larger biological material by use of screens.

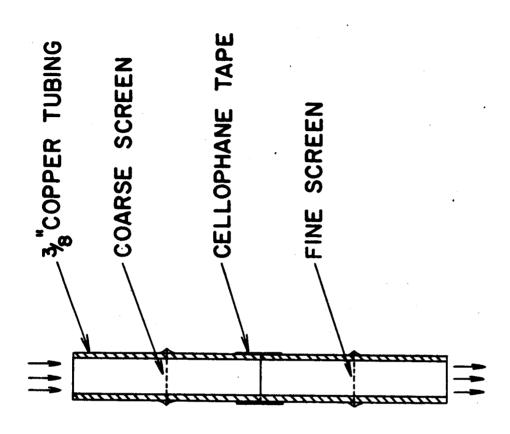
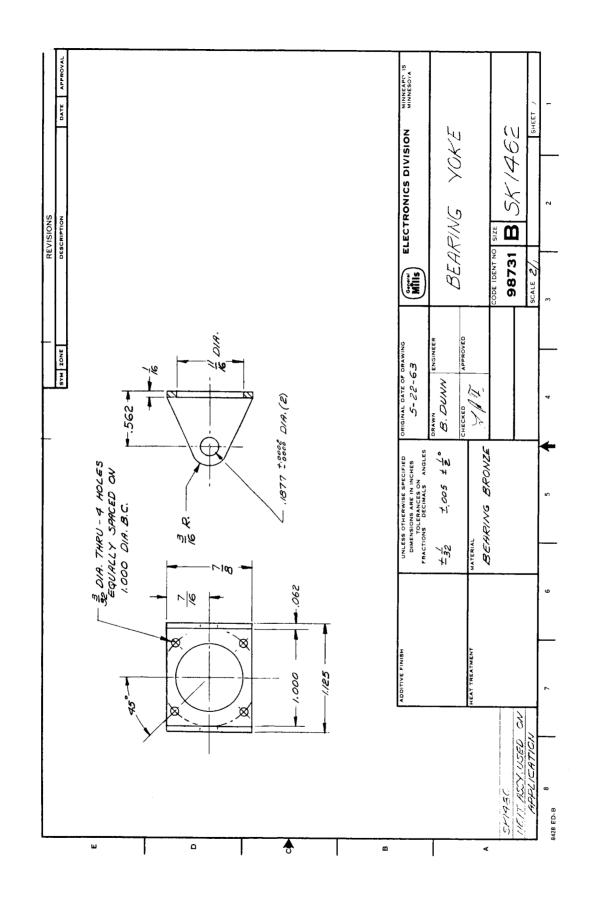
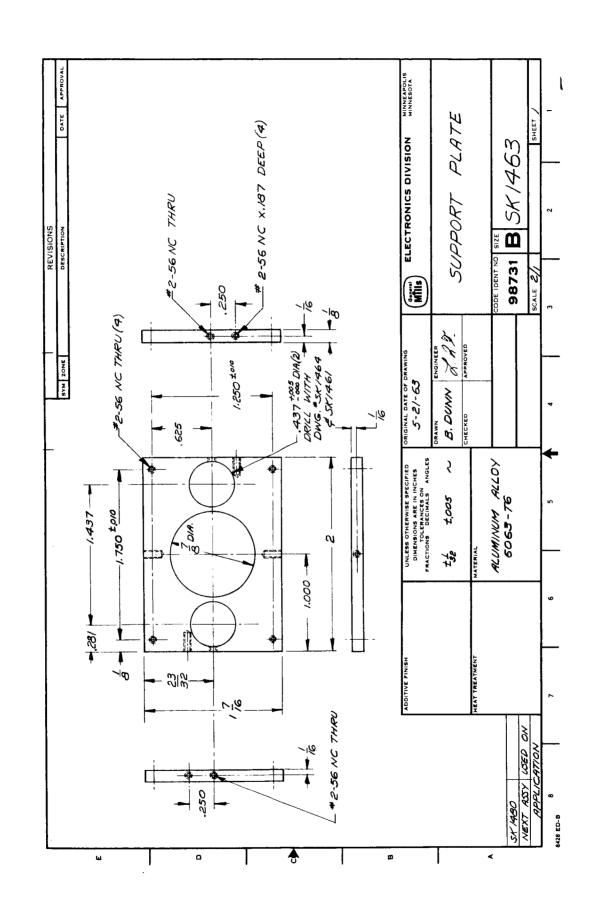
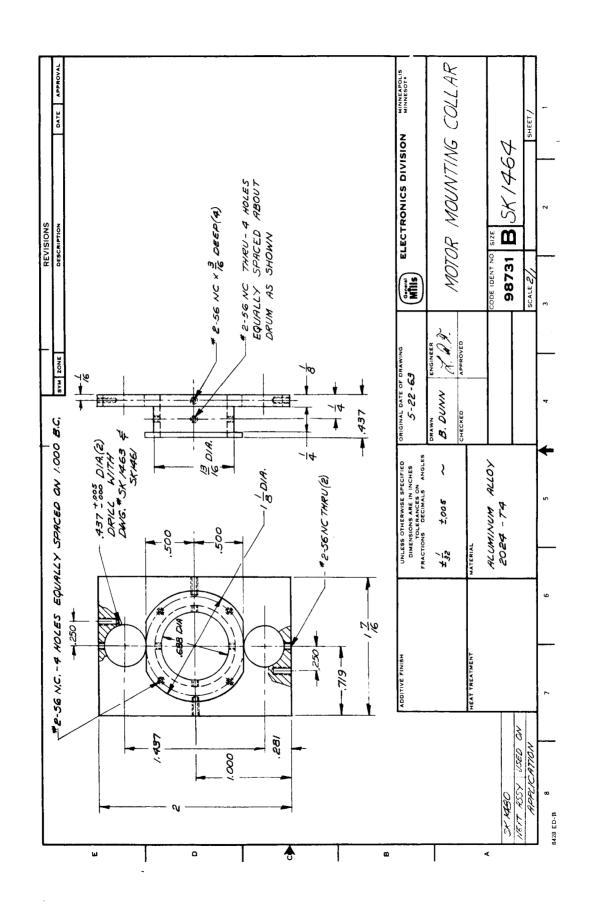


FIG. B3 SUCCESSIVE SCREEN APPARATUS FOR PARTICLE-SIZE FRACTIONATION

APPENDIX C
SHOP DRAWINGS







La Francisco

